CERTAIN PHYSICO-CHEMICAL RELATIONSHIPS IN THE POTATO TUBER

by

John Gillies, B.Sc.

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Certain Physico-Chemical Relationships in the Potato Tuber.

A Study of the Concentration and Distribution of Ascorbic Acid.

Object of the Investigation.

Potatoes to be used for seed purposes must reach a certain standard of freedom from various diseases, notably the virus diseases, severe mosaic and leaf-roll. Normally, stocks to be used for seed purposes are inspected during the growing season, with a view to grading them into stock seed categories or otherwise. This is usually a fairly laborious process and, at times, may not be really accurate. Apart from this, other stocks not previously graded as stock seed may be required for seed purposes and, once the tops of the plants have died down, there is no method whereby the presence of virus infection can be proved or disproved, except by grafting, a process which takes a few months to complete.

The object of the present investigation was to explore the connection between the biochemical activity of tubers and the incidence of virus infection. Since a principal line of attack had been suggested by earlier studies on the reducing value of the ascorbic acid in tubers, attention was directed to a more complete study of the concentration and distribution of ascorbic acid occurring under different conditions.

The correlation of biochemical activity with the incidence of virus infection would be an important step in the control of disease. The importance of such a relationship is at once obvious from a consideration of the following points. Crops could be graded scientifically and the personal error of the inspector could be eliminated. The stocks could be graded at any period of the nine months when the tubers are out of the ground instead of the very short interval of time when the plants are actively growing in the field. The method could also be used, in addition to the method used at present, if crops, which had not been inspected, were required under exceptional circumstances for seed purposes.

Previous Work.

Chemical Characteristics of the Potato Tuber.

Many investigations have been carried out dealing with chemical differences in potatoes. Intervarietal differences of a chemical nature have been the chief field of research, and a Committee of the Royal Society (1919), during the last war, undertook an extensive study of the chemical composition of potato tubers. The chief conclusion to be drawn from their work was that, the composition of the tubers was influenced by the locality in which the plants were grown. The variations were due to latitude rather than to longitude, especially as regards nitrogen content, and no intervarietal

differences are reported. Willaman and West (1925), working in America with American varieties, also found no intervarietal differences. In their work, they found that environment had a greater effect on the composition than varietal differences. Differences in composition between early and late varieties were, however, noted. Early maturing varieties had a lower percentage of dry matter but a higher percentage of ether extract, mineral matter, and nitrogen than later maturing varieties.

Coudon and Boussard (1897) investigated the dry-matter and nitrogen distribution in tubers. They divided the tuber into zones following the main physical divisions which can be seen with the naked eye. The zones were (1) Skin. (2) Cortical zone. (3) External medullary zone. (4) Internal medullary zone. They found the percentage of dry-matter to be a maximum in the cortical layer, but the percentage of nitrogen was greatest in the skin and lower in the cortical than in the medullary layer. Later, their results were confirmed by Glynne and Jackson (1920), who observed a greater percentage of dry-matter in the cortex than in the medulla. For the nitrogen content, they found the reverse.

Intervarietal differences in starch content were noted by Johnson and Boyle (1918), who also found that in any one variety, the cortex had a greater percentage of starch than the medulla, but that

locality and environment also influenced the value to a fairly large extent. The investigations of Sperling (1926) and of Dix (1923) also showed the existence of varietal differences in starch content.

It would, therefore, appear, that the fluctuations in total nitrogen, dry-matter and starch contents due to environment, render their determination unsatisfactory for the differentiation of varieties.

Various investigators, notably Artschwager (1924), and Bomer and Mattis (1924), tried to find variations in the solanine content of tubers, but the general conclusion seems to be that, although differences do exist, they vary with the age and size of the tuber and with exposure to light.

Many other classes of chemical compounds exist in the potato tuber which may be detected and estimated by chemical means. In particular, the presence of a number of different classes of enzymes has been noted. (Waksman and Davison (1926)). Of these diastase, invertase, and pectase of the carbohydrases, and tyrosinase, oxidase and peroxidase of the oxidising enzymes are the most important. A variation in diastatic power of potato juice according to the variety of potato was observed by Joszt and Starezewski (1922). Such a method necessarily involved preliminary extraction and preparation of the plant sap and is, therefore, unsuitable as a means of rapid estimation.

The oxidising enzymes have been detected and estimated by means of colour reactions. This work has been reviewed at some length by Raper (1928). He deals particularly with peroxidase, tyrosinase, polyphenolase and indophenol oxidase. Colour reactions have also been suggested by Haas and Hill (1928), for the detection of pectic substances in plant tissues, and also for the detection of amino acids and phenols (Thorpe (1927)), by the formation of coloured dyes. Probably the most important of these coloured reactions, from a practical standpoint at least, is that reported by Lauder and Robertson (1931). This is an enzyme reaction using the enzyme tyrosinase which has tyrosine for its substrate in the potato. Using p-cresol, which is similar in constitution to tyrosine, as substrate, the above investigators developed a method by which the separation of different varieties was made possible. This was done by measuring the red colour produced by the reaction after a given time in a tintometer. By this means a numerical colour value was given to each variety. These investigators found that soil type, environment, and season were without influence on the reaction. On the other hand, diseased or damaged tubers and tubers which had been greened by exposure to light gave results which were not in agreement with those obtained from healthy stock.

Separation of Healthy and Diseased Tubers.

Enzyme activity also provided a field of research for those workers interested in the separation of diseased and healthy tubers. The oxidase and phenolase activity of healthy and diseased tubers was studied by Pfankuch and Lindau (1935), but they found little difference. On the other hand, Ehrke (1935) found differences in the oxidase and peroxidase activity of healthy and "Iron-Stain" tubers. He also found differences in the glutathione and ascorbic acid contents. He found that in diseased tubers the glutathione content was greater than in healthy tubers, also that the ascorbic acid content of diseased parts was greater than that of healthy tubers.

Hey (1932) had previously claimed that, by an examination of the oxidation-reduction potentials of tubers, healthy and diseased tubers could be distinguished. The oxidation potentials were in the region of -120 m.v. and -250 m.v. respectively, for healthy and diseased tubers.

Within the last ten years vitamin-C has been the subject of much intensive study, and it is not unnatural that potatoes should have come under investigation. Most investigators were merely interested in potatoes as a source of vitamin-C, and paid little attention to either intervarietal differences or differences due to disease and other factors. Birch, Harris and Ray (1933), Ahmad (1935),

McHenry and Graham (1935) and Pett (1936) have been among the most active workers in this field. Up to the present, little has been published dealing with intervarietal differences in ascorbic acid, but Hausen (1935) has investigated the effect of vitamin-C on the growth of plants, while Ijdo (1936) has investigated the influence of fertisilers on the vitamin-C content of plants and Wachholder and Nehring (1938) have investigated the effect of manuring on the vitamin-C content of potatoes in particular. Smith and Paterson (1937) devised a rapid routine arbitrary extraction method whereby they could, in the case of many well-known varieties, distinguish between healthy tubers and those of the same variety infected with virus diseases.

The results of the last mentioned investigation were considered to be of sufficient merit to warrant further study and, for the present investigation, attention was directed to a more complete study of the occurrence of ascorbic acid under different conditions in the potato. In previous investigations the terms "presumed healthy" and "diseased stock" were necessarily related to material secured from plants which seemed in the field to be healthy or infected with virus, and in the present investigation steps were taken to have these terms defined more exactly by actual grafting experiments.

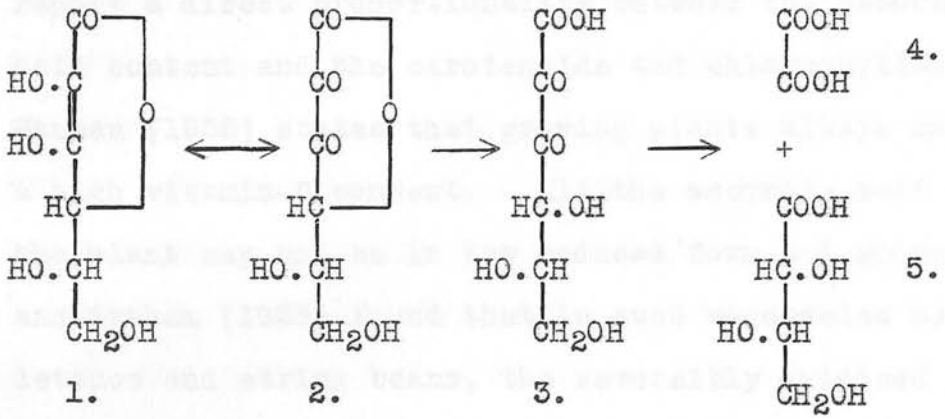
The Ascorbic Acid Content of the Potato Tuber.

Introduction - Chemistry.

Vitamin-C also called hexuronic acid or cevitamic acid, but most commonly ascorbic acid, was the first vitamin to be synthesised and to have its chemical constitution completely established. It was first obtained synthetically in 1934 by Reichstein and Grüssner (1934) after its constitution had been proved by Hirst et allia (1933).

Ascorbic acid is 3-keto-l-gulofuranolactone, and it exists as such in nature, as well as in a reversibly oxidised form called dehydroascorbic acid. The presence of both of these, givesrise to an oxidation-reduction system which may play a part in the oxidation-reduction potentials of cells, and may possibly act catalytically in certain oxidation-reduction processes. The reversibly oxidised form, dehydroascorbic acid, is only stable in very acid solution at a pH less than 5.0, and it is difficult to understand how the vitamin remains undestroyed in tissues which have an oxidising level of potential higher than that of ascorbic acid. It has been suggested that some stabilising substance is present. The dehydro or reversibly oxidised form passes readily through two other stages, the transformation occurring with increasing rapidity as the pH becomes greater than 6.0. The products of both changes are more strongly reducing than ascorbic acid itself.

The changes correspond to the transformation from dehydroascorbic acid to diketogulonic acid, also known as irreversibly oxidised ascorbic acid, at a pH of 5.5, and to the further degradation to decomposition products previously characterised as l-threonic acid and oxalic acid.



1. Ascorbic acid, 3-keto-l-gulofuranolactone
2. Dehydroascorbic acid
3. 2;3-diketo-l-gulonic acid
4. Oxalic acid
5. l-threonic acid

Bonsook, Davenport, Jeffreys and Warner (1937), were able to confirm these observations. While ascorbic acid is to be regarded as a physiological transformation product of the carbohydrates, other substances having great physiological activity have been shown to be complex compounds containing carbohydrate residues, e.g. cozymase and adenylic acid, and ascorbic acid may well be similar.

Distribution and Occurrence.

Ascorbic acid is widely distributed in nature both in plant and animal life. Giroud gives an excellent account of the occurrence of the vitamin in many varied tissues in his monograph (1938). In plant tissues Giroud, Ratsimamanga and Leblond (1935) report a direct proportionality between the ascorbic acid content and the carotenoids and chlorophylls. Hausen (1935) states that growing plants always have a high vitamin-C content. All the ascorbic acid in the plant may not be in the reduced form and McHenry and Graham (1935) found that in such vegetables as lettuce and string beans, the reversibly oxidised form was present in greater amounts than the reduced form. Tomatoes, lemon juice, etc., on the other hand are said to contain all the ascorbic acid in the reduced form. Some authors suggest that the reversibly oxidised ascorbic acid found is the result of oxidation during extraction, but it will be shown in the following pages that, at least in the potato, a proportion of the vitamin-C is originally in the oxidised form. Several authors, notably Hirst and Zilva (1933) and Fox and Levy (1936), have shown that reversibly oxidised ascorbic acid has some antiscorbutic activity.

The variation of vitamin content with maturity has been investigated by Virtanen (1936), and he finds that vitamin concentrations reach a

maximum at an early stage in growth. He also states that all factors which have an unfavourable effect on the growth of plants, such as soil acidity, excessive concentrations of phosphate, potassium, etc., lower the carotene and ascorbic acid content. McHenry and Graham (1935), also observed that on boiling certain foodstuffs in water, they obtained an increase in the ascorbic acid content. They accounted for this by assuming that the ascorbic acid was combined in some way with the protein, and that the complex was hydrolysed by the boiling water. This view was also held by Bezssonoff (1936), who suggests that, in some cases, the vitamin occurs in a methylated form which is inactive. In the cabbage he claims that as much as 30% of the vitamin is esterified. Levy (1936) also found similar results with the potato and the cauliflower.

Determination of Vitamin-C.

Mansfield Clarke and his collaborators were probably the first to record the reduction of an indophenol dye by biological material. They showed that the di-bromo substituent, 2,6-dibromophenolindophenol, was reduced by many biological fluids and living tissues. Zilva (1927) carried out much work in isolating the antiscorbutic fraction of lemon juice, and in trying to elucidate the possible constitution of the antiscorbutic factor.

Eventually, he used phenolindophenol itself in his investigations on vitamin-C. He attributed the reduction which, he found, was given by many concentrates of vitamin-C, not to vitamin-C itself but to an associated reducing and protective substance. Tillmans, Hirsch and co-workers (1932) introduced the use of 2,6-dichlorophenolindophenol for the determination of the antiscorbutic factor. In contrast to Zilva they claimed that the antiscorbutic factor itself was responsible for the reduction of the indicator. The extraction process of Tillmans and his colleagues was somewhat complex and was a qualitative guide rather than a quantitative method of analysis for the determination of the amount of ascorbic acid present in tissue. Their method, however, provides the basis for the most widely used method for the determination of vitamin C, i.e. titration against the redox dye 2,6-dichlorophenolindophenol. Most of the improvements in the method have been to modify the conditions of the reaction in order to make it more specific for vitamin C, and thus convert it into a more precise analytical method.

Several other methods have since been suggested for the determination of ascorbic acid. The most popular of these are based on colorimetric methods with, or without, a photo-cell.

Medes (1935, 1936) has worked out the colorimetric determination of ascorbic acid using

phospho-18-tungstic acid. A method based on the observation that the reduced form gives furfural on boiling with hydrochloric acid has been devised by Roe (1936), the furfural being determined colorimetrically with aniline acetate. The reduction of an azo dye by ascorbic acid has been used by Scudi and Ratish (1938) as a means of determining vitamin C, the readings being made colorimetrically. Guthe and Nygaard (1938) have described an apparatus employing the photo-cell to determine the end-point of the titration using methylene blue as indicator.

Spectrometric methods have also been tried but the apparatus required is not of a simple nature, and so this method has not found much favour. Many related substances also interfere. The pH of the solutions used also has a rather critical bearing on the absorption spectra. Chevallier and Choron (1937) have perfected a microspectrometric method using the absorption spectra in the ultra-violet from 2400 to 3000 Angstrom units.

Recently, methods claimed to be more specific than any yet introduced, have been put forward. They utilise the ascorbic acid oxidase present in most plants for the oxidation of the ascorbic acid. Tauber, Kleiner and Mishkind (1935) have devised such a method, but Neuweiler (1936) and Snow and Zilva (1938) claim that it is not entirely specific.

For the present investigation, the indophenol method was adopted, as it appeared more suited to rapid routine determinations than the other methods mentioned above.

The Indophenol Method.

The main improvements on Tillmans' method have been devised with a view to making the reaction more specific, and to get rid of interfering substances. Under the conditions used by Tillmans the method was not specific, since pyrogallol, glutathione, cysteine, catechol and tannic acid also reduced the indicator. By titration at pH 2.5-3.0 it is possible to avoid the reaction of glutathione and cysteine. This is, in effect, the modification introduced by Harris and Ray (1933). Emmerie and van Eekelen (1936) proposed the precipitation of cysteine and glutathione with mercuric acetate, but this has the disadvantage that adsorption of the vitamin may occur. (Mercuric acetate has the advantage, however, that thiosulphate, which may interfere is also removed). Using a 5% trichloracetic acid solution as their extracting medium, Birch, Harris and Ray (1933) concluded that cysteine was the only naturally occurring reducing agent which would reduce the indicator under their conditions. Millikan (1935) has since shown that in 2.5% trichloracetic acid solution, the rate of reduction of the indicator by cysteine is 350 times slower than that by ascorbic acid. Thus, under the conditions

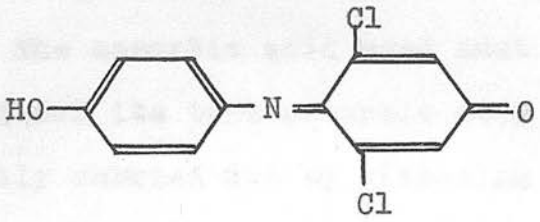
proposed by Harris and Ray (1933) the Tillmans method is, to all intents and purposes, specific for ascorbic acid. Pfankuch (1935) has further modified the method for the determination of the ascorbic acid in presence of much glutathione. It has been suggested by Cheftel and Pigeaud (1936) that by titrating at 0° C. the oxidation of the vitamin is slowed sufficiently to give more constant end-points and excessive haste becomes unnecessary. Because of the ease with which ascorbic acid may undergo reversible oxidation, van Eekelen and Emmerie (1936) consider the preliminary reduction with hydrogen sulphide a necessary step in the procedure, but Harris thinks that this is unnecessary if the extraction is carried out with reasonable speed. In order to inhibit the activity of the ascorbic acid oxidase, Musulin and King (1936) suggest the use of metaphosphoric acid along with trichloracetic acid in the extraction process.

Several investigators have adapted the indophenol indicator to colorimetric methods. Among these are Evelyn, Malloy and Rosen (1938), and Bessey (1938), who modified Evelyn's method.

When using the 2,6-dichlorophenolindophenol dye for the determination of ascorbic acid, several precautions must be observed. The dye should be free from impurities, carefully standardised and used only in fresh solution, otherwise the end-point is rather indistinct. The extraction of the ascorbic

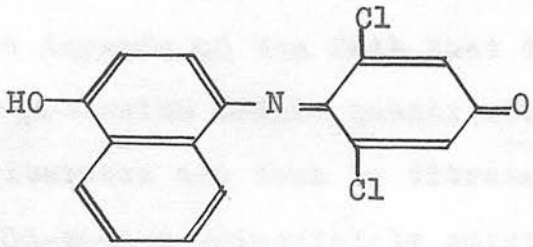
acid from the tissues should be done in an acid medium and carried out as rapidly as possible in order to reduce oxidation to a minimum, but titration at 0° C. is unnecessary.

Dye Solution. The indicator used was 0.0005 M. made up from the solid reagent supplied by Messrs. B.D.H. Ltd. This has the formula



and the sodium salt used has a molecular weight of 289.98; thus a 0.0005 M. solution is 0.0145%.

Another indicator having the same name but the following formula



is supplied by Hoffmann La Roche, Ltd. and care must be observed when making up the solution to know which compound is being used. This last compound has a molecular weight of 340 (Na salt) and thus a 0.0005 M. solution is 0.017%.

Standardisation of 2,6-dichlorophenolindophenol.

The original method used by Harris and Ray (1933) is as follows. Approximately 10 mg. of pure ascorbic acid was weighed out accurately and dissolved in 2% trichloracetic acid and made up to 200 ml. in a standard flask. 10 ml. of this solution were used in each titration against the indophenol. Thus the ascorbic acid equivalent for 1 ml. of indophenol can be found. The ascorbic acid used must also be standardised for its true ascorbic acid content. This is easily carried out by titrating the ascorbic acid solution against a standard N/100-iodine solution. It should be noted that one molecule of ascorbic acid reduces two atoms of iodine.

Another method of standardising the indicator has recently been introduced by Menaker and Guerrant (1938), which depends on the fact that the indophenol will oxidise potassium iodide quantitatively to iodine. The iodine liberated can then be titrated with a standard N/100-sodium thiosulphate solution. The authors claim that this method is more accurate than that just described. The chief advantages of the method are that the sodium thiosulphate solution which is being used continually for standardisation remains stable for a long time once it has reached equilibrium. The end-point is also much sharper than that obtained by the previous method.

Yet another method has been worked out by

Lorenz and Arnold (1938) whereby ferrous ammonium sulphate is used as the basic standard for the 2,6-dichlorophenolindophenol solution. A solution of Mohr's salt (1g. per l.) is titrated with the indophenol in the presence of oxalic or metaphosphoric acid.

In the present investigation, the method of Menaker and Guerrant was compared with that of Harris and Ray. It was found that the former method was as accurate as Harris' method and when frequent standardisation of the dye is required, as in the present investigation, it has the decided advantage of taking up less time since the standard sodium thiosulphate can be kept for some time.

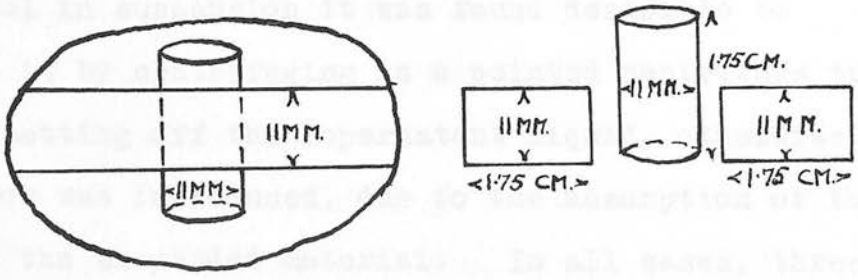
For the standardisation, 25 ml. of the 0.0005 M. dye solution were pipetted into a 50 ml. conical flask and 1 g. (10 ml. of 10% solution) of potassium iodide added along with 2 ml. dilute sulphuric acid (4N.). The flask was then shaken to facilitate the oxidation of the potassium iodide. The liberated iodine was titrated immediately with standard N/100 sodium thiosulphate using the usual starch indicator. It has been established that 1 ml. of 0.01 N iodine solution is equivalent to 0.88 mg. of ascorbic acid; consequently, 1 ml. of 0.01 N sodium thiosulphate is also equivalent to 0.88 mg. ascorbic acid, and thus the ascorbic acid equivalent of the indophenol can be found.

Laboratory Technique.

During the course of the investigation, various types of tissues were examined. Several methods of extraction were used, depending on the type of tissue involved, and the particular purpose for which it was required.

(a) Tuber Tissue. "Quick Extraction Process."

In order to obtain a representative sample of the tuber tissue, three cores were taken from a tuber. Cores were taken and a portion cut off with parallel knives fixed at 1.75 cm. apart. Two cores were taken with a cork borer 11 mm. in diameter, one longitudinally, the other transversely. Two pieces were then cut from the longitudinal core and one from the transverse core by means of the parallel knives. Each piece weighed about 2 g. The following diagram shows the method of cutting the cores.



For the "Quick Extraction" method, the dimensions of the cores must be strictly adhered to as the method is essentially comparative.

The three cores so obtained were at once placed in 10 ml. of a 2% trichloracetic acid solution and shaken for one minute. The supernatant liquid was then decanted and titrated with the standardised

0.0005 M. indophenol. The titration value so obtained is the figure used when comparing the relative ascorbic acid contents of tubers, and is hereinafter called the "indophenol value" of the tuber.

(b) Tuber Tissue. "Complete Extraction Process." Three cores were taken from the tuber as described in (a). The cores were now transferred as quickly as possible to a mortar, and about half the final volume of 2% trichloracetic acid (in this case 25 ml.) was added. About 8 g. of pure iron-free sand were then added, and the whole ground into a pulp. The extract was now decanted off and filtered through glass wool into a 50 ml. standard flask. Glass wool was chosen because it was found to be the quickest filtering medium giving, at the same time, a fair separation of suspended material. If there was much material in suspension it was found desirable to remove it by centrifuging in a pointed centrifuge tube and pipetting off the supernatant liquid, otherwise an error was introduced, due to the adsorption of the dye on the suspended material. In all cases, three extractions were found to be desirable in order to remove the bulk of the ascorbic acid. The three extracts were all filtered into the standard flask which was then filled up to the mark and an aliquot (usually 5 ml.) taken for titration.

(c) Sprouts, Stems and Leaves. With sprouts, the

available weight of tissues was much smaller, and a micro-burette was used for the titrations, but the method of extracting the ascorbic acid was exactly the same as in section (b). Many sprouts are deeply coloured and a separate technique had to be adopted for the titration of their extracts. This is described in section (d).

(d) Coloured Solutions. Certain coloured solutions provided an additional problem, since the colour present nearly always obscured or masked the end-point. Emmerie and van Eekelen (1934) suggested the use of mercuric acetate as a precipitant, but McHenry and Graham (1935) found this method unsatisfactory since only about 70% of the ascorbic acid was recovered. They, in turn, introduced a method depending on the fact that most plant pigments are insoluble in chloroform, whereas the indophenol indicator is more soluble in chloroform than in water. The technique adopted in this investigation was essentially that of Olliver (1938). The ascorbic acid solution was placed in a 15 ml. narrow pointed centrifuge tube along with 2 ml. chloroform. Stirring was effected by a stream of oxygen-free carbon dioxide or nitrogen. Centrifuging may be required to break the emulsion in the lower layer. Addition of the indicator was continued until the chloroform layer, after being mixed with the extract layer, developed a definite pink colour. It was most important that the

extract and ~~in~~ the indicator be well mixed before the chloroform and aqueous layers were mixed. If the indicator came into direct contact with the chloroform layer a pink colour, not discharged by ascorbic acid, might result. A rough titration was carried out first to give an approximate idea of the titration value, and thus save time in the final titration.

(e) Dehydroascorbic Acid. It was found that in the potato tubers, there was always a certain amount of dehydroascorbic acid present, though this amount varied considerably throughout the season, and so this had also to be taken into account when determining the total ascorbic acid content of the tissue. In the present instance, the total ascorbic acid was determined by taking a known volume of the extract obtained in (b), transferring it to a 50 ml. conical flask and passing H_2S for a period of 15 minutes. At first, trouble was experienced, due to excessive frothing when the gas was passed, but this was overcome by the addition of 2 ml. of isopropyl alcohol. After passing the H_2S, the flask was tightly stoppered and allowed to stand overnight. In the morning, the excess H_2S was driven off with a stream of oxygen-free nitrogen until there was no positive test for H_2S with lead acetate paper. This usually required a period of about 10-15 minutes. The indophenol titration was now carried out in the usual manner. Ken and Watson (1936) give a very good method for the purification of the

nitrogen to be used in such circumstances. The nitrogen is first passed through three wash-bottles containing the following reagents (1) alkaline pyrogallol (2) sulphuric acid (3) trap. The gas is next passed through an electrically heated combustion tube filled with copper turnings to another wash-bottle containing an alkaline glucose solution along with methylene blue. This last solution is made up by adding a 1% solution of methylene blue drop by drop to a hot 1% glucose solution in N/10 NaOH until a faint blue colouration is obtained. The temperature is then raised until the solution becomes colourless.

Freezing of Cores and its Effect on the Ascorbic Acid Content.

Normally, when it is required to determine the ascorbic acid content of a large number of tubers, the process is rather tedious, so an attempt was made to improve the method by a freezing process. When plant cells are frozen, the cell walls become disrupted so that on thawing the contents should be more easily reached by the extracting agent, in this case 2% trichloracetic acid.

First of all, the freezing was done with solid carbon dioxide. A hole was bored in the solid block and into this the cores, taken in the usual manner, were dropped and left for a minute or two. (Very little time was required for the cores to

freeze). When the cores were taken out and allowed to thaw, the tissue was found to be spongy and to be losing a considerable amount of moisture, thus showing that an irreversible change had taken place in the structure of the potato tissue.

The frozen cores were treated in various ways. Some being ground alone; others were ground with sand before and after thawing. In all cases there was little difference in the state of division of the tissue as compared with the ordinary extraction method, and, in fact, it had one very serious draw-back, as will be seen later.

Liquid air was next tried as the freezing medium. The cores were tied up in a small muslin bag and the whole dipped into the liquid. On grinding the frozen cores, a great number of unbroken cells were visible under the microscope. By far the most important point, however, from the point of view of the present investigation, was the loss of vitamin-C on freezing.

The ascorbic acid content of various frozen cores of a few varieties was determined by the normal indophenol method to find out the effect of freezing. First of all, the determination was carried out with the unfrozen tissue by the method (b) already described, as a control. A comparable set of cores was now frozen as described above, and the ascorbic acid content again determined. It was found that it

was very much reduced, in fact it was only about one-sixth of the amount contained in the fresh tissue (See Table 1).

Table 1.

Ascorbic acid in mg. per 100 g. tissue after grinding cores with sand. Frozen cores compared with unfrozen.

Variety.	Unfrozen.	Frozen.
Arran Chief	6.51	1.05
Up-to-Date	8.96	3.20
Sharpe's Express	7.78	1.55
President	6.67	1.21

There was a possibility that the ascorbic acid might have been oxidised by contact with the liquid oxygen, but when the cores were frozen by placing them in a test-tube and dipping the tube into the liquid air, the vitamin content was still low. Thus the effect of the intimate contact with the oxygen seemed to be of little account. Some of the extract was treated in the usual manner with H_2S to determine the amount of reversibly oxidised ascorbic acid. In this case the ascorbic acid content was about double the first value, but was still low compared with the fresh material. (See Table 2). Thus it would seem as if some of the vitamin is irreversibly oxidised or destroyed in some way.

Table 2.

Increase in ascorbic acid content in mg. per cent of frozen tissue after treatment with H_2S.

Variety.	Before H_2S	After H_2S
Sharpe's Express	1.55	3.72
President	1.21	4.43

It is worthy of note that a pure solution of ascorbic acid in water and in 2% trichloracetic acid experienced no reduction of the reducing value on freezing in a similar manner, but when frozen in the presence of tuber tissue a fall in the ascorbic acid content was found. (In the following experiment the Great Scot was frozen in a test-tube, while the Majestic was frozen in muslin). A pure solution of ascorbic acid gave the following result when frozen, and the results in Table 3 are based upon it.

	Unfrozen.	Frozen.
15 ml. soln.	10.78 ml.	10.55 ml.

Table 3.

Indophenol titration values when cores were frozen in presence and absence of free ascorbic acid. (15 ml. = 10.78 ml. Indo.).

Variety.	Sample.	Frozen.	Unfrozen.	Theoret.	Loss.
Great Scot	8.17g.		2.90 ml.		
	15 ml. + 9.49g.		13.23 ml.	13.92 ml.	0.69 ml.
	7.93g.	0.70 ml.			
	15 ml. + 8.60g.	9.98 ml.		11.57 ml.	1.59 ml.
Majestic	10.92g.		3.73 ml.		
	15 ml. + 10.86g.		13.45 ml.	14.15 ml.	0.70 ml.
	8.89g.	0.64 ml.			
	15 ml. + 7.37g.	9.08 ml.		11.05 ml.	1.97 ml.

Conclusion. Freezing did not effect any obvious improvement in the rupture of the cell walls of the potato tuber as compared with the ordinary grinding method with sand, and the latter was just as quick. When frozen, an irreversible change is produced in the tissue as shown by the fact that the tissue, on thawing, is spongy and loses a considerable amount of moisture.

A marked lowering of the indophenol titration value caused by the process of freezing and thawing is probably associated with the structural breakdown of the tissue, brought about by the formation of ice crystals which may act directly causing mechanical damage or indirectly by concentrating the aqueous phase. It is the latter which is responsible for the change in living muscle when it is frozen. This disintegration may well lead to the liberation into the free liquid of enzymes capable of inactivating or oxidising the vitamin. Mills (1935) found very similar results when liver was frozen. When frozen, a certain amount of vitamin-C was lost, and on prolonged storage in the frozen state, further destruction occurred. Bracewell found that storage at low temperatures had no effect on the antiscorbutic potency of apples, but the stability of the vitamin has been shown to vary in different media. The loss in antiscorbutic activity may also be connected in some way with the denaturation of the protein. It

is worthy of note that Izumrudova (1935) found that potatoes stored at 2.5° C. gradually lose vitamin-C, but that frozen potatoes retain their potency. In all probability, his experiments were carried out with whole tubers and not with cores as in this investigation which would account for the large losses found in the present work.

Diurnal Variation in the Ascorbic Acid Content. Leaves and Stems.

At one stage in the present investigation, samples had to be taken from plants growing in the field and it was thought desirable, in order to make the results as comparable as possible, that attention should be paid to the variation in the ascorbic acid content at various times of the day and night. As will be seen from the following results, the hour of sampling was found to have a large effect on the ascorbic acid content, especially in the leaves.

Experimental Procedure. Plants of a few varieties were specially marked for this work and, at intervals, groups of three or four leaves were taken from the heads of these plants and immediately placed in 25 ml. of 2% trichloracetic acid. The weight of the leaves taken was afterwards found by difference. For the determination of the ascorbic acid content, the leaves were ground up with trichloracetic acid and the determination carried out as previously described for coloured solutions (page 21), after centrifuging to

GRAPH I.

RELATION BETWEEN ASCORBIC ACID CONTENT OF LEAVES OF KERR'S PINK AND TIME OF DAY.

get rid of the suspended material.

The varieties used were Kerr's Pink and Epicure and samples were taken over a period of 24-48 hours and, in one case, at intervals of one hour for a period of eight hours.

This work was carried out about the middle of the month of June when the tubers were just beginning to form and the plants were about 18" high. As far as could ascertained, all the plants used for the investigation were healthy.

Experimental Results. Case 1. The leaves of Kerr's Pink were sampled at hourly intervals from 8.0 a.m. to 5.0 p.m. G.S.T. and the results given below are calculated on a fresh-weight basis. (See Table 4, and Graph 1).

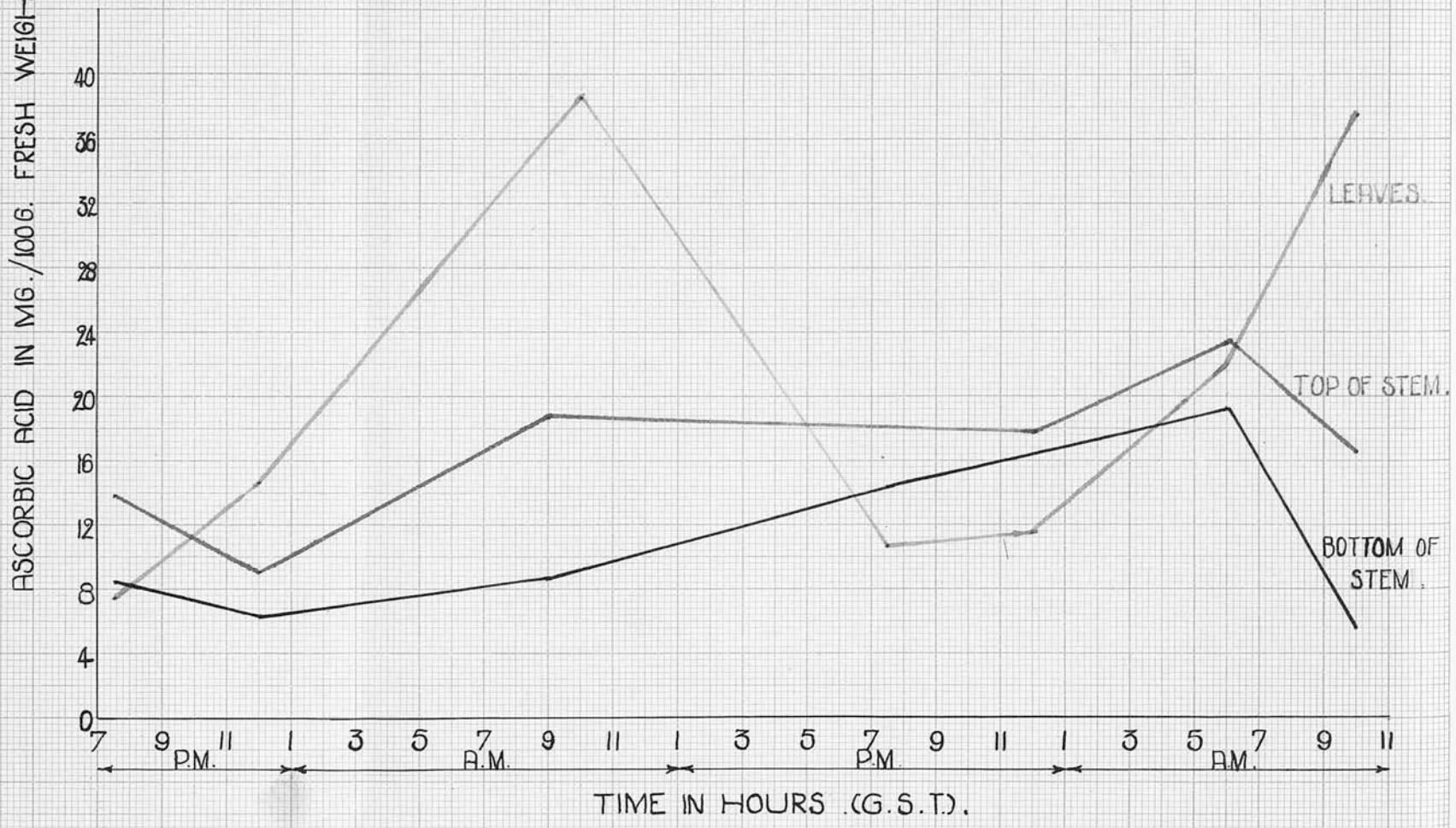
Table 4.

Ascorbic acid content of leaves of Kerr's Pink on a fresh-weight basis, expressed in mg. per 100 g.

Time	Ascorbic Acid
8.0 a.m.	8.21
9.30 "	10.47
10.30 "	10.92
11.30 "	8.55
12.30 p.m.	10.95
1.30 "	8.34
2.30 "	8.53
3.30 "	8.73
5.0 "	8.83

GRAPH 2.

RELATION BETWEEN ASCORBIC ACID CONTENT OF LEAVES AND PETIOLES OF KERR'S PINK OVER 48 HOURS.



From these results it is obvious that the highest ascorbic acid concentration occurs between 10 a.m. and 12 noon. The drop at 11.30 a.m. is rather difficult to explain, but it is interesting to note that, Barton-Wright and McBain (1933) have given a very similar type of curve for the diurnal variations in the total nitrogen content of the laminae of healthy varieties expressed on a fresh-weight basis.

Case 2. The above figures only gave the variations during nine hours out of the twenty-four, and it was thought that, if samples were taken at longer intervals, the small hourly fluctuations would be smoothed out and a better picture of the translocation, if any, obtained. The results of this investigation are embodied in Table 5, and Graph 2.

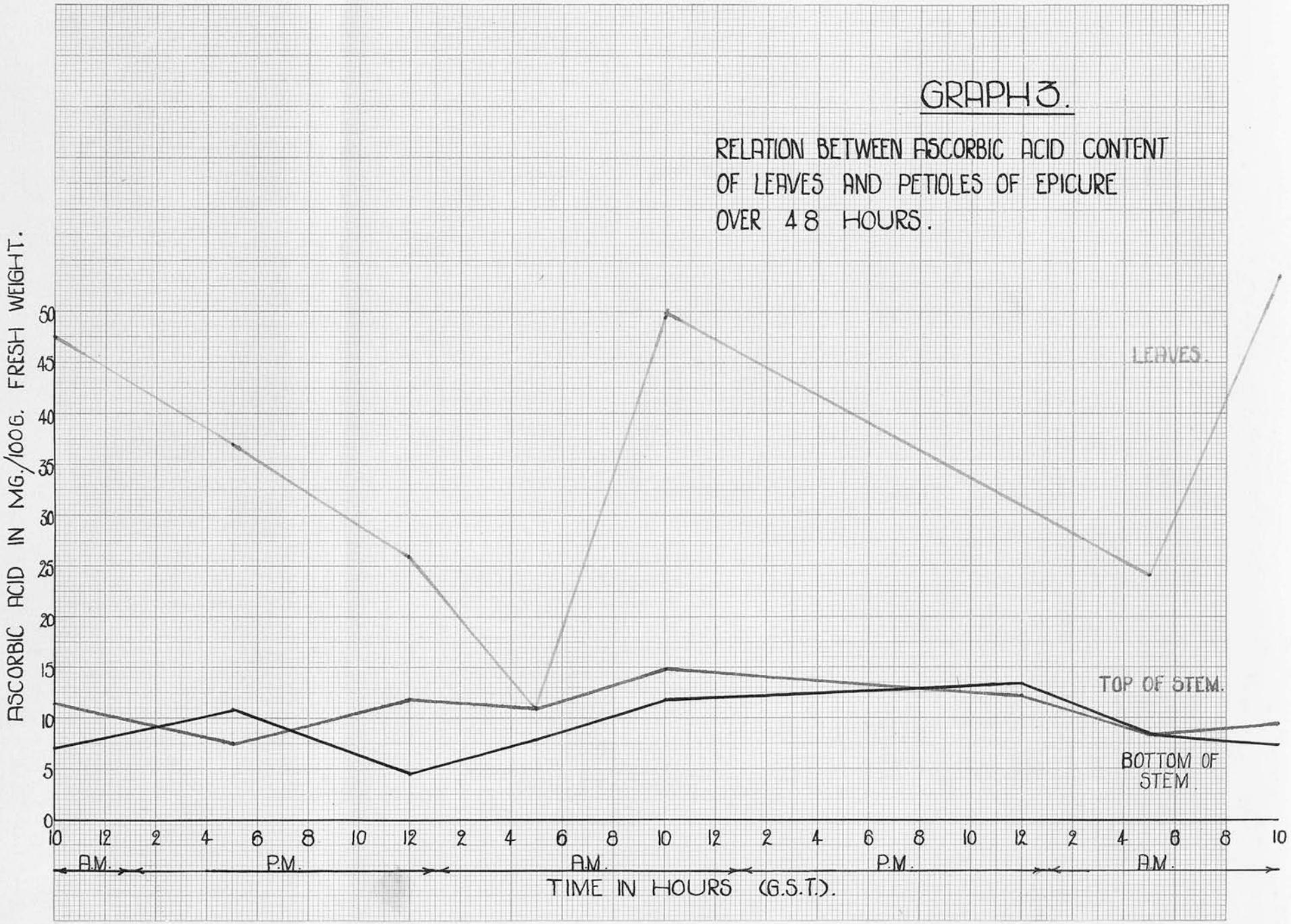
Table 5.

Ascorbic acid content of leaves and petioles of Kerr's Pink over a period of time expressed on a fresh-weight basis as mg. per 100 g.

Time (G.S.T.)	Laminae	Top of Stem	Bottom of Stem
7.30 p.m.	7.53	13.86	8.30
12.0 "	14.80	9.11	6.19
10.0 a.m.	38.39	18.86	8.63
7.30 p.m.	10.75	---	14.27
12.0 "	11.42	17.92	---
6.0 a.m.	21.68	23.11	15.23
10.0 "	37.61	16.72	5.72

GRAPH 3.

RELATION BETWEEN ASCORBIC ACID CONTENT OF LEAVES AND PETIOLES OF EPICURE OVER 48 HOURS.



When these results are plotted on a graph the irregularities visible on the previous graph are smoothed out, and it is obvious that the concentration of ascorbic acid is at its highest about 10 a.m. (G.S.T.). This graph also helps to give a picture of the distribution of the ascorbic acid down the stem of the plant. The top of the plant always has a greater concentration than the lower part of the stem, but round about midnight, the concentration in the leaves falls below that of the stem. The variations in the stem are not nearly so great as the fluctuations in the leaves, but it is not the case that a stem minimum means a leaf maximum. This is to be expected when it is remembered that translocation is going on from the leaves all the time, helping to raise the concentration in the stem.

Case 3. The variety Epicure was also investigated with a view to finding out the variation in the ascorbic acid content throughout the twenty-four hours. The results are given in Table 6, and Graph 3. Samples were taken at intervals over a period of 48 hours, and the figures quoted are based on the fresh-weight of the material.

Table 6.

Ascorbic acid content of leaves and petioles of Epicure over 48 hours expressed on a fresh-weight basis as mg. per 100 g.

Time (G.S.T.)	Laminae	Top of Stem	Bottom of Stem
10.0 a.m.	47.36	11.42	6.98
5.0 p.m.	37.11	7.49	10.78
12.0 "	25.54	11.65	4.60
5.0 a.m.	11.07	11.08	7.70
10.0 "	49.45	14.59	11.80
12.0 p.m.	---	12.20	13.29
5.0 a.m.	24.12	8.20	8.25
10.0 "	48.52	9.37	7.67

The results of this experiment in the main, bear out those of the last. The differences between the top and the bottom of the stem are not quite so clear-cut. The concentration of ascorbic acid in the stem never rises above the concentration in the leaves, as was the case with Kerr's Pink.

Conclusion. The concentration of ascorbic acid in the leaves and stems of the potato varies considerably throughout the day, but reaches a maximum in the early forenoon. The variation in the stem is not nearly so great as in the leaf. The fact that the concentration in the leaves is always greater than in any other part of the plant, would seem to suggest that the leaves are the source of ascorbic acid in the plant, and that ascorbic acid is, therefore, a product of photosynthesis. This

subject will be discussed further in a subsequent section. The path of the ascorbic acid down the stem is also seen when it is noted that the foot of the stem has a maximum, following a maximum of, and almost coinciding with a minimum of the leaves. The actual time lag is, however, rather difficult to discern.

Relation between Ascorbic Acid content of Leaves and Tubers During the Growing Season.

For the purpose of this experiment, two plots were laid down in different localities, one having a pH of 6.7 and the other a pH of 6.2. At the former three varieties were planted. They were Arran Pilot, Arran Consul and Dunbar Cavalier. As well as healthy tubers, tubers infected with virus diseases, namely, severe mosaic and leaf-roll, were planted. For the purposes of comparative sampling, the leaves nearest the growing tips of certain numbered plants were taken along with the corresponding tubers from the same plants. In order to make sampling as uniform as possible, the leaves, etc. were always collected at 10 a.m. and the ascorbic acid determinations carried out as soon thereafter as possible. Sampling was started about the beginning of June, and continued at intervals until the end of the growing season.

The results for each variety are given separately, and are all expressed in mg. per 100 g. of tissue. (Fresh-Weight). The following letters

are used to distinguish healthy and diseased material.

E.H. Healthy Plants.

E.S.M. Plants infected with severe mosaic.

E.L.R. Plants infected with leaf-roll.

Table 7.

Ascorbic acid content of healthy and diseased leaves and tubers at different dates of sampling, in mg. per 100 g. fresh-weight.

(1) Arran Pilot

Date	Leaves			Tubers		
	E.H.	E.S.M.	E.L.R.	E.H.	E.S.M.	E.L.R.
13/6	15	-	-	10	-	-
6/7	77	55	65	28	32	27
17/8	53	-	32	31	32	18
9/9	-	-	-	20	18	23

(2) Dunbar Cavalier

Date	Leaves			Tubers		
	E.H.	E.S.M.	E.L.R.	E.H.	E.S.M.	E.L.R.
14/7	83	92	63	28	27	22
21/8	27	62	46	22	29	33
26/9	-	-	-	22	23	25

(3) Arran Consul

Date	Leaves			Tubers		
	E.H.	E.S.M.	E.L.R.	E.H.	E.S.M.	E.L.R.
20/7	30	35	17	14	15	15
21/8	16	33	20	24	35	29
14/9	-	-	-	19	18	19

The average figure for the leaves of all three varieties is 48.3 mg. ascorbic acid per 100 g. of tissue, and the comparable figure for the tubers if 24.9 mg. ascorbic acid. Thus, the concentration in the leaves is about double that in the tuber and, in some cases it is much higher still.

In the second locality, seven varieties were planted, all of which were healthy. The sampling and the analysis were carried out in the same way as before. Table 8 gives the concentration of ascorbic acid in mg. per 100 g. fresh-weight in the leaves, and Table 9 the comparative values for the tubers.

Table 8.

Ascorbic acid content of the leaves of healthy plants at different dates of sampling in mg. per 100 g. fresh-weight.

Variety	4/7	26/7	31/8
Arran Pilot	120	101	59
Great Scot	84	76	54
Kerr's Pink	-	75	51
Arran Banner	-	90	60
Redskin	-	46	60
Majestic	-	49	52
Dunbar Cavalier	-	62	66

Table 9.

Ascorbic acid content of the tubers of healthy plants at different dates of sampling in mg. per 100 g. fresh-weight.

Variety	4/7	26/7	31/8	14/9
Arran Pilot	27	14	30	17
Great Scot	17	25	26	18
Kerr's Pink	-	25	31	14
Arran Banner	-	20	34	22
Redskin	-	21	24	14
Majestic	-	21	30	14
Dunbar Cavalier	-	21	24	14

Conclusion.

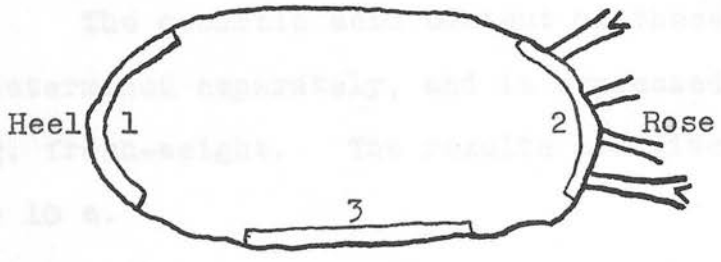
The concentration of the ascorbic acid in the young tuber varies with the stage of development of the tuber. In every case, a maximum value was reached on, or before, the middle of August. For "Early" and "Second Early" varieties, the maximum was reached rather earlier than in the other varieties, but this is to be expected when it is remembered that "Early" varieties reach maturity before the "Late" varieties, so that the maximum corresponds to the same stage of growth for each variety. The position of the maximum is not so clear-cut in Tables 8 and 9, as in Table 7, but the statement given above still holds good.

The maximum concentration of ascorbic acid in the tubers is followed by a sharp fall as the

haulms ripen and die down. The leaves at all stages in the growth have a much higher concentration of ascorbic acid than the tubers. This leads to the view that the ascorbic acid is synthesised in the leaves or, at any rate, in the leaves, we have the source of ascorbic acid in the plant, and the tuber is only a storage organ for this substance. This subject of the source of the ascorbic acid will be gone into more fully later, however.

Distribution of Ascorbic Acid in the Potato Tuber.

The following experiments were undertaken when the distribution of the ascorbic acid between tuber and sprout was being examined. In the first instance, various sections of the outer layer were examined. These were about 1/8 inch thick, and were cut as shown in the accompanying sketch.



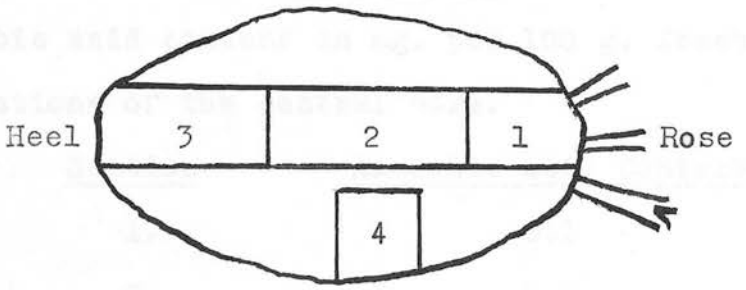
The tissue was weighed and pulped with 2% trichloracetic acid and sand in the usual manner, and the ascorbic acid content determined as mg. per 100 g. tissue fresh-weight. The results are given in Table 10.

Table 10.

Ascorbic acid content in mg. per 100 g. fresh-weight of sections of the outer layer of the potato tuber.

Section	Ascorbic Acid Content
1	6.7
2	7.7
3	6.5

The internal tissue of the tuber was next examined. For this purpose, cores were taken in the normal manner and cut into sections, as shown in the sketch.



The ascorbic acid content of these sections was determined separately, and is expressed as mg. per 100 g. fresh-weight. The results are given in Table 10 a.

Table 10 a.

Ascorbic acid content in mg. per 100 g. fresh-weight of sections of cores from the internal tissue.

Section	Ascorbic Acid Content
1.	10.4
2.	8.5
3.	8.5
4.	9.1

This seemed to give an indication of a gradient in the central core, so it was decided to investigate this further. For this purpose, the central core was divided into six portions as shown, which were pulped in the usual manner. The results are given in Table 10 b.

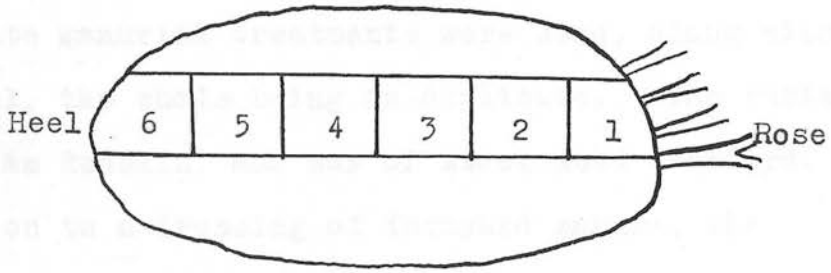


Table 10 b.

Ascorbic acid content in mg. per 100 g. fresh-weight of sections of the central core.

Section	Ascorbic Acid Content
1.	8.1
2.	7.6
3.	7.1
4.	6.8
5.	5.5
6.	4.3

It should be noted that these results were obtained after sprouting had begun, so that this may not be the normal distribution in the tuber.

Conclusion.

The concentration of ascorbic acid at the surface varies rather indefinitely, but it is

greater at the rose than at the heel end. In the deeper tissue, it is greater at the rose and lower at the heel, and, in fact, seems to follow through quite a consistent gradation.

Ascorbic Acid Content with Relation to Manuring.

For the purpose of this experiment, nine separate manurial treatments were used, along with a control, the whole being in duplicate. The variety used was Redskin, and was of stock seed standard. In addition to a dressing of farmyard manure, the following treatments were given.

Plot 1. A mixture of Ammonium Sulphate 2 parts
Potassium Sulphate 2 parts
Superphosphate 3 parts

Applied at the rate of 10 cwt. per acre.

2. A mixture of Superphosphate 6 parts
Potassium Sulphate 4 parts

Applied at the rate of 10 cwt. per acre.

3. A mixture of Ammonium Sulphate 4 parts
Superphosphate 6 parts

Applied at the rate of 10 cwt. per acre.

4. Ammonium Sulphate applied at 10 cwt. per acre.

5. A mixture of Potassium Nitrate 4 parts
Superphosphate 6 parts

Applied at the rate of 10 cwt. per acre.

6. Potassium Nitrate applied at 6 cwt. per acre.

7. Superphosphate applied at 6 cwt. per acre.

8. A mixture of Ammonium Sulphate 3 parts
Superphosphate 3 parts
Potassium Sulphate 3 parts

Applied at the rate of 10 cwt. per acre.

9. A mixture of Ammonium Sulphate 4 parts
Potassium Sulphate 4 parts

Applied at the rate of 8 cwt. per acre.

10. Control. No artificials.

The tubers were harvested at the end of September, stored at a temperature of 8-10° C. for a period of three weeks, and the ascorbic acid content determined as previously described. Three determinations were made on material under each treatment, and the averages used for comparison. The results are given in mg. per 100 g. fresh-weight.

Table 11.

Ascorbic acid content of Redskin under different manurial treatments in mg. per 100 g. of tissue fresh-weight.

Treatment	Ascorbic Acid Content			Average	
1.	15.5	13.5	14.3	14.43	
2.	19.9	17.0	12.2	16.07	
3.	11.9	16.1	17.2	15.07	
4.	16.5	13.5	14.3	14.77	Mean
5.	15.7	16.5	13.9	15.37	15.3
6.	16.0	16.7	13.1	15.27	
7.	17.3	17.4	15.3	16.67	
8.	14.7	16.5	15.8	15.67	
9.	13.5	16.6	13.9	14.67	
10.	15.0	16.8	14.3	15.37	

At first sight, the plots which received a large dressing of ammonium sulphate would seem to give a lower ascorbic acid content than the others, but the difference is not sufficient to be significant. Virtanen (1936) states that an adequate fertilisation increases the percentage of vitamin-C. He found that excessive concentrations of phosphate, potassium, etc., lowered the vitamin-C content, but that any adequate fertilisation gave an almost constant value for the ascorbic acid content. Apart from these extremes, no significant differences were reported. Wachholder and Nehring (1938) report that farmyard and artificial manures gave no differences in the ascorbic acid content in particular varieties of potatoes. On the other hand, Ijdo (1936) makes some rather interesting observations on the ascorbic acid content of spinach. He concluded that both larger amounts of nitrogen and larger amounts of potassium resulted in greater vitamin-C contents. The vitamin-C content increased but little at low nitrogen levels and as a result of the addition of potassic fertilisers, whereas at high nitrogen levels a large increase could be observed. A potassium deficiency had the effect of a nitrogen excess and a potassium excess acted like a nitrogen deficiency.

Dehydroascorbic Acid in Potatoes.

It has previously been mentioned that ascorbic acid may exist in the plant in the reversibly oxidised form as well as the ordinary reduced form. This holds good for the potato tuber as well as for other plants. McHenry and Graham (1935) found that, in certain vegetables, notably lettuce and string beans, there is more ascorbic acid present in the reversibly oxidised form than in the reduced form. On the other hand tomatoes, lemon juice, etc. are said to have all their ascorbic acid in the reduced form. Hirst and Zilva (1933) have shown that dehydroascorbic acid has at least some antiscorbutic activity despite its difference in structure from the reduced ascorbic acid. Stone (1937) discusses the dehydroascorbic acid content of the potato tuber. He shows that, after the action of ascorbic acid oxidase, almost all the ascorbic acid present in the potato is in the reversibly oxidised form.

For the determination of the dehydroascorbic acid in the potato tuber, the extraction is carried out in the same manner as for the reduced ascorbic acid, but before the titration with the 2,6-dichlorophenolindophenol, the extract must be reduced with H_2S and the H_2S subsequently removed in a stream of nitrogen as described on page 22

From previous work by Smith and Paterson (1937) it was indicated that, although immediately

after lifting the ascorbic acid present in the tuber was almost all in the reduced form, it was possible that a certain portion might exist in the reversibly oxidised form after a period of storage. This question was, therefore, examined more fully and it has been found that the amount of dehydroascorbic acid, as measured by the H_2S-reduction of the extract does, in fact, increase with storage.

In the first instance, tubers of a few varieties were lifted and the dehydroascorbic acid content determined at once. These results are given in Table 12 as mg. per 100 g. of tissue (fresh weight), and were obtained in September, 1939.

Table 12.

Reduced and Total (Reduced + Reversibly Oxidised) Ascorbic Acid in Tubers given as mg. per 100 g. tissue (fresh weight).

Variety	Reduced Ascorbic Acid	Total Ascorbic Acid
Kerr's Pink	14.1	14.0
Redskin	14.1	14.4
Majestic	14.4	14.8
Arran Pilot	16.6	16.2
Great Scot	17.5	17.3
Arran Banner	22.3	24.3
Totals	99.0	101.0
Average	16.5	16.8

Thus, shortly after harvesting, there is, on the average 0.3 mg. dehydroascorbic acid per 16.5 mg. ascorbic acid, corresponding to 1.8% of the total ascorbic acid in the tuber, in the reversibly oxidised form. The experimental error, however, is greater than these values, so only a very small amount, if any, of the ascorbic acid would appear to be in the reversibly oxidised form at this stage.

From preliminary observations it had been found that, after storage, this value was altered considerably so it was decided to check the dehydroascorbic acid content at intervals during the dormant season. This was done after (a) four weeks' (b) two months' (c) four months' (d) six months' storage. The results are given in Table 13, and are expressed in mg. per 100 g. tissue on a fresh weight basis. Both the reduced (Red.) and total (Tot.) ascorbic acid contents are given, and the amount of dehydroascorbic acid present is given by the difference.

Before being examined, the tubers were kept in a dark storehouse within which an almost constant temperature of 5° C. prevailed.

The first part of the table gives the results generally, while the second part gives the results when strictly comparable sets are compared.

It should, perhaps, be emphasised, that the tubers examined on lifting (Table 12) were not from the same source as those used here to examine the effect of storage.

Table 13.

Reduced and Total (Reduced + Reversibly Oxidised) Ascorbic Acid in Tubers given as mg. per 100 g. tissue (fresh weight) after varying periods of storage.

Storage	4 weeks		2 months		4 months		6 months	
Variety	Red.	Tot.	Red.	Tot.	Red.	Tot.	Red.	Tot.
Arran Consul	16.4	19.3	8.7	10.3	4.9	8.8	5.2	7.9
Arran Chief	-	-	10.8	12.0	5.7	8.0	6.4	8.5
Di-Vernon	-	-	8.8	10.4	6.2	7.5	7.5	11.3
President	-	-	-	-	6.7	5.6	5.6	8.3
Dunbar Cavalier	13.9	17.2	11.3	12.0	7.1	7.9	6.3	9.7
Majestic	15.4	18.3	8.6	10.0	7.4	8.9	5.5	7.2
Doon Star	-	-	12.7	16.8	7.6	8.5	5.3	9.5
Epicure	-	-	-	-	7.7	8.9	5.2	7.0
Dunbar Standard	-	-	-	-	7.7	8.9	7.5	10.2
King Edward	23.4	24.5	18.8	21.2	8.1	9.2	5.6	8.0
Duke of York	-	-	-	-	8.3	10.1	7.4	9.8
Up-to-Date	19.2	21.2	-	-	8.5	10.2	8.0	9.8
Arran Banner	-	-	8.6	12.5	8.7	9.8	7.8	10.0
Gladstone	-	-	-	-	9.3	10.7	6.4	7.6
Redskin	26.7	28.3	12.2	13.8	9.7	12.6	5.1	9.5
Golden Wonder	19.4	20.0	10.9	12.7	9.8	11.5	6.6	9.2
Great Scot	20.1	24.4	10.4	12.0	-	-	7.2	9.0
Kerr's Pink	22.4	24.4	12.9	15.9	-	-	9.5	11.0
Totals	176.9	197.6	134.7	159.6	123.4	147.1	118.1	163.5
Average	19.66	21.96	11.2	13.3	7.7	9.2	6.6	9.1
Average Increase	2.30		2.10		1.50		2.50	
Percentage Dehydro.	10.5		15.8		16.3		27.5	

Dehydroascorbic acid content when strictly comparable sets are compared.

6 Varieties Percentage Dehydro.	9.9	11.3	13.3	33.7
8 Varieties Percentage Dehydro.	10.9	13.3	-	-
10 Varieties Percentage Dehydro.	-	15.9	19.3	33.0
16 Varieties Percentage Dehydro.	-	-	16.3	30.0

Conclusion.

Ascorbic acid exists in the potato in the oxidised as well as in the reduced form, but the proportion which exists at any given time depends chiefly on the period of storage of the tubers. Just after the tubers have been lifted, the proportion is small, but it increases rapidly during the first month of storage and after that, though an increase still takes place, it is not so rapid as at the start until near the end of the storage period when sprouting is accompanied by another large increase.

The actual increment or the actual dehydroascorbic acid content is worth noting. It would seem to be fairly constant after the first month, so that the proportional increase is due rather to a fall in the ascorbic acid content than to an increase in the amount of the dehydroascorbic acid present. The actual source of the dehydroascorbic acid is not known with certainty, but it is probable that it is formed at the expense of the reduced form and is, itself, further oxidised to the irreversibly oxidised form as the time of storage increases. The dehydroascorbic acid would seem to be in equilibrium with both the reduced and the irreversibly oxidised ascorbic acid.

Ascorbic Acid and Storage.

The effect of storage on the ascorbic acid content can also be seen from Table 13. The following Table is a resumé of the results obtained

from Table 13, as far as it concerns the effect of storage on the ascorbic acid content.

Table 14.

Ascorbic Acid Content of Tubers after varying periods of storage expressed as mg. per 100 g. tissue (fresh weight).

Storage	4 weeks	2 months	4 months	6 months
General Av.	19.66	11.2	7.7	6.6
Av. of 6 Vars.	19.20	11.8	7.8	5.7
Av. of 8 Vars.	19.70	11.7	-	-
Av. of 10 Vars.	-	11.1	7.5	6.1
Av. of 16 Vars.	-	-	7.7	6.3

Thus we see that the amount of ascorbic acid present after six months' storage is only about one-third of that present after one month's storage.

In a recent paper, Zilva and Barker (1940) working with the variety King Edward found that the time of lifting, with respect to maturity, had an important bearing on the ascorbic acid content during the storage period. In general, tubers lifted when immature, had lower concentrations after a period of storage than those allowed to mature normally. They also report losses on storage similar to those given above.

Ascorbic Acid and Disease.

A few workers have noted the effect of virus diseases on the ascorbic acid content, among those being Virtanen (1936) and Hausen (1935). Smith and Paterson (1937) noted an increase in the ascorbic acid content of potato tubers with the incidence of mosaic and leaf-roll viruses. From a practical standpoint such an increase, if consistently reproducable, would have immense value in separating healthy from diseased stock.

The following work was carried out in order to find if such differences did exist, and if so, whether they were constant. The greatest difficulty of this type of work is the obtaining of tubers guaranteed free from infection or, at least, tubers infected with known viruses. This can only be done after prolonged grafting experiments, and only in a few cases was tested material actually used in the present instance. For the most part, stocks placed in the Stock Seed categories were taken as healthy material, and the infected material was picked from material which seemed in the field to be diseased. Previous work done by Smith and Paterson (1937) had been carried out using a quick extraction method, and for the present investigation it was decided to carry out first the full extraction process to see whether similar results were obtained.

Table 15 gives the reduced ascorbic acid content of several varieties after varying periods of storage. Healthy (E.H.) tubers, and tubers infected with severe mosaic (E.S.M.) are compared. The results are expressed as mg. per 100 g. tissue (fresh weight).

Table 15.

Ascorbic Acid Content of Healthy and Diseased Tubers after varying periods of storage expressed as mg. per 100 g. tissue.

Storage	1 month		4 months	
Variety	E.H.	E.S.M.	E.H.	E.S.M.
Arran Pilot	18.8	23.3	9.8	9.5
Dunbar Cavalier	13.9	26.4	7.1	7.5
Arran Consul	16.4	16.8	4.9	5.3
Great Scot	20.1	21.3	8.5	8.9
Kerr's Pink	22.4	29.8	11.8	9.3
Redskin	26.7	24.7	9.7	10.1
Majestic	15.4	18.4	7.4	7.2
King Edward	23.4	28.3	8.1	9.3
Golden Wonder	19.4	17.5	9.8	5.7
Up-to-Date	19.2	20.6	8.5	9.9
Arran Banner	15.0	25.0	8.7	7.7
Duke of York	24.0	22.0	8.3	11.7
Gladstone	22.4	19.3	-	-
Catriona	24.9	31.6	-	-
Doon Star	19.0	21.0	7.6	6.9
Arran Chief	15.4	16.1	-	-
Sharpe's Express	24.1	25.2	-	-

After one month's storage, thirteen out of the seventeen varieties examined showed an increase in the concentration of ascorbic acid in the virus infected tuber, the others showed a decrease. After four months' storage, no definite conclusions could be drawn. Only six of the varieties showed an increase in the ascorbic acid content when infected with virus. These results were not very conclusive, so it was decided to see whether the total ascorbic acid content of the tubers would give more consistent results. These results are given in Table 16 as mg. per 100 g. of tissue (fresh weight).

Table 16.

Total Ascorbic Acid Content of Healthy (E.H.) and Diseased (E.S.M.) Tubers after varying periods of storage expressed as mg. per 100 g. tissue (fresh weight).

Storage	1 month		4 months	
Variety	E.H.	E.S.M.	E.H.	E.S.M.
Arran Pilot	32.6	28.8	-	-
Dunbar Cavalier	17.2	26.5	7.9	8.0
Arran Consul	19.3	21.0	8.8	7.1
Great Scot	24.4	22.4	-	-
Kerr's Pink	24.4	31.8	-	-
Redskin	28.9	25.9	12.6	12.1
Majestic	18.3	21.3	8.9	7.9
King Edward	24.5	28.3	9.2	14.7
Golden Wonder	20.0	17.5	11.5	9.8
Up-to-Date	21.2	24.8	10.2	10.4
Doon Star	-	-	8.5	11.2
Duke of York	-	-	10.1	12.9
Arran Banner	-	-	9.8	11.7

The total ascorbic acid content after four months' storage does not give any more consistent results than the concentration of the reduced form, and the results of the determinations after one month's storage are no better. The discrepancy after four months' storage might be explained by the fact that the majority of the tubers by this time are beginning to sprout, so that their internal metabolism

would be active, but this does not explain the irregularity of the results after only one month's storage.

To see if any explanation could be found, it was decided to go over the quick extraction method again with tubers which had been stored for about one month. Here, the actual "Indophenol value" of the tuber cores is given, this being the volume of "Indophenol Reagent" in millilitres required to oxidise the ascorbic acid in a trichloracetic acid extract of a constant weight of tuber tissue. (See page 19). The results of this investigation are given in Table 17.

Table 17.

Comparison of the "Indophenol Values" of cores of healthy tubers and tubers infected with severe mosaic.

Variety	"Indophenol Value" Healthy	"Indophenol Value" Mosaic
King Edward	0.96 ml.	1.21 ml.
Kerr's Pink	1.07	1.27
Doon Star	0.46	0.51
Great Scot	0.55	0.55
Eclipse	0.55	1.05
Sharpe's Express	1.14	1.37
Redskin	0.63	0.91
Up-to-Date	0.90	1.04
Golden Wonder	0.60	0.60
Arran Chief	0.45	0.52
Catriona	1.32	1.27
Majestic	0.44	0.41
Arran Banner	0.89	0.94
Arran Pilot	0.72	0.70
Dunbar Cavalier	0.65	0.61
Di-Vernon	0.56	0.87
Arran Consul	0.46	0.54

At first sight, there does not seem to be any relation between the incidence of the mosaic virus and the "Indophenol Value" of the infected material, but if all those varieties which show an increase in the titration value of more than 0.1 ml. are grouped together and the varieties showing no increase

or, at any rate, an increase of less than 0.1 ml. are grouped together, an interesting comparison can be made. To appreciate the significance of this grouping, it is necessary to refer to the distribution of certain of the viruses through the various varieties.

Scott (1938) and Cockerham (1939) have grouped the varieties as follows :-

Group 1 - Varieties lethally necrotic to virus A and virus X.

Group 2 - Varieties lethally necrotic to virus A but non-lethal to virus X.

Group 3 - Varieties lethally necrotic to virus X but non-lethal to virus A.

Group 4 - Varieties non-lethal to virus A and virus X.

Picking out the varieties for which experimental results are given, we find that the following varieties fall into Groups 1 and 2. King Edward, Kerr's Pink, Doon Star, Great Scot, Eclipse, Sharpe's Express, Redskin, Up-to-Date.

None of the common varieties is lethally necrotic to virus X alone, and Group 3, therefore, may be ignored. To Group 4 belong the varieties Golden Wonder, Arran Chief, Catriona, Majestic, Arran Banner, Arran Pilot, Dunbar Cavalier, Di-Vernon, Arran Consul.

Of the varieties falling into Groups 1 and 2, only Doon Star and Great Scot fail to show a marked increase in the "Indophenol Value" with the incidence of disease, and in Group 4 the only

variety which shows a significant increase is Di-Vernon. In other words, of seventeen varieties examined, only three are exceptions to the general behaviour of the other members of the above grouping, based on the distribution and the occurrence of certain of the viruses.

It should be noted that there are several factors to be borne in mind when considering these results. Firstly, although the health material used was of Stock Seed standard, some of it may have carried primary infection, and secondly, as it was obtained from various growers, it may not all have been lifted at the same time. Barker and Zilva (1940) have shown that the time of lifting has an important bearing on the concentration of ascorbic acid during the storage period, and this could quite conceivably upset the values, since the differences which were being measured are relatively small. Further, the stipulated increase of 0.1 ml. in the "Indophenol Value" is purely arbitrary, and may not be giving a true representation of the differences found. These points will be more fully dealt with in the general discussion.

After the results of the preceding experiment, it was thought possible that plants noted in the field to be infected with leaf-roll might also show some varietal differences with regard to the increase in their "Indophenol Value" between the "healthy" and "diseased" states. Table 18 gives the results obtained.

Table 18.

Comparison of the "Indophenol Values" of cores of healthy tubers and tubers infected with leaf-roll.

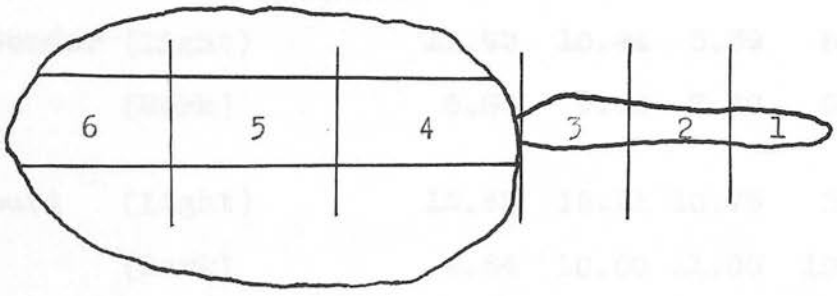
Variety	"Indophenol Value" Healthy	"Indophenol Value" Leaf-roll
Kerr's Pink	1.07 ml.	0.99 ml.
Gladstone	0.93	1.02
Eclipse	0.55	0.53
Majestic	0.44	0.37
Arran Banner	0.89	1.04
Doon Star	0.46	0.49
Up-to-Date	0.90	0.91
Golden Wonder	0.60	0.67
King Edward	0.96	1.62
Redskin	0.63	0.80

From the above results no definite conclusions can be drawn. Some of the varieties show an increase in the "Indophenol Value", while others show a decrease. The only one showing a really significant increase is King Edward with an increase of 0.66 ml. The next is Redskin which has a much lower increase of 0.17 ml. It is worthy of note that King Edward is the only one of the above varieties falling into Group 1, i.e. it is the only one lethally necrotic to both virus A and virus X. Whether this fact is of importance or not, cannot be determined at the present juncture, as further work would require to be carried out on the other varieties comprising Group 1, before any generalisation could be made, but it would seem

rather strange that the only variety, of the ten examined, which is irregular, should belong to this group.

Distribution of Ascorbic Acid in Tubers Grown in Light and Dark.

For the investigation of this feature, about twelve tubers of each of the following varieties were placed in trays exposed to the light, and another corresponding twelve tubers were kept in trays in the dark. The varieties were Golden Wonder, King Edward, Great Scot, Arran Pilot. After the tubers were sufficiently sprouted, samples were taken from the tubers as shown in the accompanying sketch, six samples being obtained from each tuber.



All the tips, shoot centres, etc. for one experimental set were combined for the determination which was carried out in the usual manner (page 20) using an automatic micro-burrette for the sprout determinations because of the small quantities involved. The weight of each portion taken was noted, and thus the concentration of ascorbic acid in mg. per 100 g. of tissue could be found. The results are summarised in Table 19.

Table 19.

Comparison of the Ascorbic Acid contents of Tubers and Sprouts Grown in the Light and the Dark, given as mg. per 100 g. tissue.

Variety		Ascorbic Acid Content in mg. per 100 g.					
	Section	1.	2.	3.	4.	5.	6.
Golden Wonder	(Light)	11.93	10.44	5.39	8.28	5.99	5.95
	(Dark)	5.83	9.40	7.68	8.28	6.74	6.02
King Edward	(Light)	12.63	15.31	10.75	8.78	6.86	6.78
	(Dark)	9.64	10.00	11.00	10.50	5.10	7.10
Great Scot	(Light)	22.15	15.62	11.67	8.80	7.19	6.77
	(Dark)	9.77	14.65	10.42	9.77	7.58	7.73
Arran Pilot	(Light)	10.27	16.66	12.18	9.12	6.88	5.19
	(Dark)	9.88	15.76	15.33	11.85	9.07	9.97

Conclusion. In every case, when the tuber had been exposed to light, there was an increase in the concentration of ascorbic acid from the heel end of the tuber to the middle or tip of the sprout. When the tuber had sprouted in the dark, the concentration gradient was not so steep and not quite so regular, and the highest concentration was, with one exception, found in the middle of the sprout. The concentration of ascorbic acid in all cases was greater in the middle and tip of those sprouts exposed to the light than in the corresponding positions of those developed in the dark. The following table of average values from Table 19 gives a very good idea of the nature of the gradient in tubers growing in light and dark.

	Tuber			Sprout		
	Heel	Middle	Rose	Bottom	Middle	Tip
Light	6.2	6.7	8.8	10.0	14.5	14.3
Dark	7.7	7.1	10.1	11.1	12.5	8.8

There is not sufficient data available here to decide whether the observed differences are due to transfer of ascorbic acid from tuber to sprout, or to the synthesis of ascorbic acid in the growing tip, but this point will be dealt with in the next section of the investigation.

Source of Ascorbic Acid in the Growing Sprout.

This is actually an extension of the previous part of the investigation. Eight tubers of the following four varieties were weighed and placed in trays exposed to the light, and a corresponding set of tubers were weighed and kept in the dark. The varieties were Great Scot, King Edward, Golden Wonder, Redskin. At the start of the experiment, the ascorbic acid content of the four varieties was determined, and from this value the total amount of ascorbic acid in each tuber was found. After the tubers had sprouted sufficiently, the sprouts of each tuber were cut off, weighed and the ascorbic acid content determined. The tubers were also weighed and their ascorbic acid content determined. From this it was possible to calculate how much ascorbic acid had been lost from the tuber since sprouting had begun. Knowing the amount present in the sprouts obtained from the weighed tuber, it is thus possible to see whether, or not, any ascorbic acid has been synthesised. The results are given in Table 20, Section A. and were obtained after the tubers had been allowed to sprout for approximately 12 weeks. In Section B. of Table 20, the corresponding values are given for the total ascorbic acid, i.e. reduced+ reversibly oxidised ascorbic acid, in one variety, Redskin.

Table 20.

Comparison of the Ascorbic Acid contents of Tubers and Sprouts grown in the Light and in the Dark, showing the loss of ascorbic acid since sprouting began.

A.

Variety		Tuber 14/2/40			Tuber 6/5/40			Diff. in Vit.C	Sprout 6/5/40			Loss of Vit.C
		W.	C.	w.	W.	C.	w.		W.	C.	w.	
Great Scot												
Light	1	300	10.6	31.8	256	9.9	25.3	6.5	6.94	20.9	1.45	- 5.0
	2	200	10.6	21.2	165	9.9	16.3	4.9	5.85	22.6	1.32	- 3.6
Dark	1	258	10.6	27.3	174	9.2	16.0	11.3	35.00	6.2	2.18	- 9.1
	2	296	10.6	31.4	212	9.9	21.0	10.4	33.00	7.3	2.40	- 8.0
King Edward												
Light	1	222	7.4	16.4	185	6.4	11.8	4.6	3.40	28.5	0.97	- 3.6
	2	181	7.4	13.4	150	6.5	10.3	3.1	2.81	28.1	0.79	- 2.3
Dark	1	198	7.4	14.7	163	7.4	12.1	2.6	7.37	11.9	0.88	- 1.7
	2	168	7.4	12.4	140	8.4	11.8	0.6	5.09	17.3	0.88	+ 0.3
Golden Wonder												
Light	1	225	9.8	22.1	180	9.1	16.4	5.7	4.41	22.0	0.97	- 4.7
	2	147	9.8	14.4	102	9.2	9.4	5.0	2.27	19.4	0.44	- 4.6
Dark	1	225	9.8	22.1	166	8.2	13.6	8.5	13.80	8.3	1.14	- 7.4
	2	160	9.8	15.7	115	8.6	9.9	5.8	11.33	9.2	1.04	- 4.8
Redskin												
Light	1	210	11.0	22.1	172	11.0	18.9	3.3	4.04	22.8	0.92	- 2.4
	2	259	11.0	28.5	204	10.6	21.6	6.9	4.67	18.9	0.88	- 6.0
Dark	1	220	11.0	24.2	140	8.9	12.5	11.7	16.40	7.0	1.14	-10.6
	2	212	11.0	23.3	140	10.6	14.8	8.5	21.30	8.5	1.81	- 6.7

B.

Redskin												
Light	1	210	13.8	29.0	172	12.7	21.9	7.1	4.04	37.1	1.50	- 5.6
	2	259	13.8	35.7	204	12.4	25.3	10.4	4.67	32.1	1.50	- 8.9
Dark	1	220	13.8	30.1	140	11.3	15.8	14.3	16.40	11.3	1.85	-12.4
	2	212	13.8	29.3	140	9.2	12.9	16.4	21.30	14.6	3.12	-13.3

where W. = Total weight of tissue.

C. = Conc. of ascorbic acid in mg. per 100 g. tissue.

w. = Total weight of ascorbic acid present in tissue in mg.

Conclusion. The results obtained here bear out the conclusions drawn from Table 19 that there is a greater concentration of ascorbic acid in sprouts grown in the light than in sprouts grown in the dark. The actual weight of ascorbic acid present in the sprouts grown in the dark is, however, greater due to the greater weight of sprout tissue. Over the three months during which sprouting has been going on, there has been a net loss of ascorbic acid, indicating that no vitamin C is being synthesised at this stage in the life-cycle. The ascorbic acid, therefore, must be passing from the tuber to the sprout. The concentration of the ascorbic acid in the sprouts grown in the light is much greater than in the sprouts grown in the dark. This may be because of the increased area of respiration in the etiolated sprouts causing more vitamin C than normal to be used in plant processes.

The exceptionally great losses in total ascorbic acid are worth noting. These are probably explained by the fact that there appears to be less dehydroascorbic acid in the sprouted tuber, since no exceptional differences are to be found in the amounts of the reduced form.

Ascorbic Acid Oxidase.

There was a possibility that all the reducing value found in the tuber tissue might not be due to ascorbic acid. The increases in the indophenol titration with diseased material may be due to the presence of some other indophenol reducing substance other than ascorbic acid.

Studies in the enzymic determination of ascorbic acid were begun by Szent-Györgyi (1928) who concluded that the oxidation of ascorbic acid by plant tissues was due to peroxidase acting through the intermediary of a phenolic compound, the phenol being first oxidised by peroxidase to the quinone which, in turn, oxidised the ascorbic acid presumably without the intervention of a second enzyme. Later, (1930, 1931) he modified this view and postulated the existence of a specific "hexoxidase" which was responsible for the oxidation of the ascorbic acid. Zilva (1934) showed that apples contained an enzyme, distinct from and unconnected with peroxidase which was capable of reversibly oxidising vitamin C, and which he considered to be similar in nature to Szent-Györgyi's hexoxidase.

The indophenol titration method for the determination of vitamin C is at best only approximate for the method assumes in general, that the only substance present in the tissues with a reduction potential lower than that of 2,6-dichlorophenolindophenol, is ascorbic acid. The capacity of the tissues to reduce the indicator is taken to represent their

ascorbic acid content.

Tauber and Kleiner (1935) were the first to use ascorbic acid oxidase for estimating its substrate in natural sources, and their method has been perfected by Srinivasan (1937).

Ascorbic acid oxidase is widely distributed in nature, and it is being increasingly used for the determination of ascorbic acid in biological material.

In the present investigation, the oxidase was prepared from potatoes according to the method used by Srinivasan (1936) with several minor modifications.

Preparation of the Enzyme.

About 800-1000 g. of washed and dried tubers were passed twice through a mincer, and the juice pressed out from the resulting pulp. Due to the action of the tyrosinase present, the pulp gradually turned brown due to the formation of melanin. The extract was centrifuged at 3500 r.p.m. for 20 mins. to deposit any starch etc. suspended in the liquid. Glacial acetic acid was added to give a final concentration of 1% and the extract placed in a refrigerator overnight. The precipitate which settled out was centrifuged off the next morning, and the supernatant liquid saturated with ammonium sulphate. The liquid was allowed to stand overnight and, in the morning, the fine precipitate which appeared was separated by centrifuging at

3500 r.p.m. for 30 mins. The precipitate was now washed with 25 ml. cold saturated ammonium sulphate solution and again centrifuged. The precipitate was next taken up in 25 ml. water and the solution once again saturated with ammonium sulphate and allowed to stand overnight when it was again centrifuged. The precipitate was now dissolved in 25 ml. water, put into a cellophane bag from which the inorganic salts were removed by dialysis. This final solution contains the required enzyme, and the solution at this stage is usually yellowish-brown in colour. The exact amount of enzyme present was not of major importance since it was required to act qualitatively with the ascorbic acid rather than quantitatively.

In the first instance, the enzyme was incubated with a pure solution of ascorbic acid. Five ml. of the ascorbic acid solution was put into a series of 50 ml. flasks along with 1 ml. of the enzyme extract obtained above. The flasks were now kept at 34° C. for varying periods of time, at the the end of which 10 ml. of trichloracetic acid were added to stop the enzyme action. The solutions were then titrated with indophenol in the usual manner. The results are given in Table 21.

Table 21.

Action of Ascorbic Acid Oxidase on a pure solution of Ascorbic Acid after varying periods of time.

Control	5 ml. ascorbic acid soln.		=	13.20 ml. Indo.
1 ml. Ext. +	5 ml. ascorbic acid soln.	30 mins.	=	1.60 ml. Indo.
1 ml. Ext. +	5 ml. ascorbic acid soln.	60 mins.	=	0.22 ml. Indo.
1 ml. Ext. +	5 ml. ascorbic acid soln.	90 mins.	=	0.20 ml. Indo.

From these results it is obvious that, after incubating for one hour, all the ascorbic acid seems to have been used up. In the succeeding experiments, all the tests were carried out using 60 mins. as the period of incubation.

When a trichloracetic acid extract is made from a potato tuber the pH of the resulting solution is about 2.5 and in this medium the enzyme is inactive. The optimum pH for the enzyme reaction is about 5.0 (Srinivasan (1936)). To get the required pH sodium carbonate solution of a strength of 4 g. per l. was added. Normally 16-18 ml. of this solution were required, giving a final volume of solution approaching 40 ml.

Ten ml. of the extract from six cores were used in each determination. No enzyme was added to the control, but the sodium carbonate solution was added in every case. After the incubation period of 60 mins. 10 ml. of 2% trichloracetic acid were

added to inactivate the enzyme again, and the indophenol titration carried out in the usual manner. Both healthy and diseased material was examined. The results of the investigation are given in Table 22.

Table 22.

	E.H.		E.S.M.	
Variety	Control Vol. Indo.	After Incub. Vol. Indo.	Control Vol. Indo.	After Incub. Vol. Indo.
Catriona	3.80	0.22	3.72	0.22
King Edward	2.30	0.20	2.96	0.22
Up-to-Date	3.42	0.24	3.50	0.20
Majestic	1.95	0.18	1.88	0.20

Conclusion. As far as can be judged from the above sets of figures, the whole of the indophenol titration value, both for healthy and diseased material, seems to be due to the ascorbic acid present. This only holds true, of course, if the oxidase is specific for vitamin C, and does not oxidise any other reducing substances present. The residual value may, at first, seem rather large, too large in fact to be neglected, but when it is remembered that the volume of solution is in the neighbourhood of 40 ml. this does not seem too great a value for the blank titration.

Discussion of Results.

It is evident that ascorbic acid is present in the potato tuber, and that it can be detected and estimated by means of the redox dye 2,6-dichlorophenol-indophenol under certain defined conditions. By means of this indicator, a quantitative determination of the ascorbic acid present can be made. The concentration of ascorbic acid is not by any means constant. It varies from tuber to tuber, but these variations are not as great as the variations caused by variety or disease. For any one variety only an average value can be given at any specific time, but individual tubers come so close to this average value that to all intents and purposes it represents the value for that variety when healthy.

The greatest factor affecting the concentration of ascorbic acid in the potato tuber would seem to be the stage in growth, (or storage), at which the determination is carried out. The content for any one variety is constantly changing from month to month, so that the results of different investigators cannot be readily compared. Despite this, it should be possible for different investigators to show the same order for varieties. For example, (Table 13), King Edward always has a higher ascorbic acid content than Majestic. This is confirmed by Zilva and Barker (1940).

Because of the difference in the ascorbic acid concentrations of different varieties of tubers,

it is possible to separate tubers of similar tuber characteristics. In actual practice, the normal application would be to identify two varieties with similar tuber characteristics, the name of one, or both, being known. For example, King Edward and Dunbar Cavalier, Arran Pilot and Sharpe's Express can be separated in this manner. This method may be extended, since any variety with a high value can be distinguished from one with a low value. There are, however, limitations to this method, for it would be almost impossible to identify, with any great accuracy, a tuber which might belong to any of the known varieties. When trying to identify a particular variety, it would also be necessary to have a control of tubers of the suspected variety or varieties. This might not always be successful, since storage conditions might not be the same in all cases.

The effect of disease must also be taken into account when trying to separate varieties, for it will be seen from the foregoing results that, in certain varieties, virus diseases are accompanied by an increase in the concentration of ascorbic acid. This increase in the ascorbic acid content with the incidence of disease is of practical importance also. Potato breeders have been trying for a long time to find a simple method for testing for the presence of mosaic diseases and the ascorbic acid content could be used for such a test in certain cases enumerated in the text.

For this test, however, material definitely free from virus is required as a control. In the present investigation, tubers from Stock Seed collections were occasionally found to give a higher ascorbic acid content than the average, and the inference was that these tubers were not free from disease. On the other hand, tubers supposed to contain mosaic virus, i.e. tubers obtained from plants observed in the field to be infected, were encountered which gave results similar to the corresponding healthy tubers. The results obtained with the virus infected material, though rather irregular, seem to be due to more than chance, but no definite statement can yet be made on this subject. In certain varieties, there would seem to be a connection between the incidence of the mosaic virus and the concentration of ascorbic acid in the tuber. Certain of the irregularities in the differences between diseased and healthy material may be explained by the fact that, some of the so-called healthy material may not be virus free, or by the effect of storage which has been shown to be very large. The actual time of harvesting may also play an important part in the final concentration of ascorbic acid in the tuber, as has been shown by Zilva and Barker (1940) for the variety King Edward. From the results, it would seem that any further investigation in this field would require carefully controlled glass-house experiments extending over a period of at least three years.

A possible explanation for the increased ascorbic acid content noted is as follows. In the normal healthy plant a certain level of ascorbic acid is necessary for growth promoting purposes, as in the human body. Any slight excess or deficiency in this amount only leads to a slight increase or decrease in the growth of the plant. When the plant is infected with virus the normal amount of ascorbic acid attempts to counteract the disease, but there is insufficient present for this as well as for the normal growth of the plant, so more of the vitamin is synthesised, and a concentration greater than that normally found, is set up. This theory is given some support by the fact that Lojkin (1936) showed that pure ascorbic acid completely inactivated purified preparations of tobacco mosaic virus, provided that atmospheric oxygen was present. This hypothesis may be adapted to give two complimentary explanations for the grouping of the varieties given on page 57.

(a) The lethal necrosis of Groups 1 and 2 is due to the increase of the ascorbic acid taking place when they are infected and, therefore, such an increase does not take place in the varieties of Group 4, thus allowing them to be infected.

(b) The varieties of Group 4 being non-lethal to virus A are all infected with the virus and, therefore, show no increase in the "Indophenol Value" when "diseased" material is examined. It should

be noted that all the material seemed to be "healthy" or "diseased", as the case may be. This explanation implies the assumption that virus-free material would give a lower "Indophenol Value" than infected material.

The source of the extra ascorbic acid in the diseased material might conceivably be in a virus protein. With the breakdown of this protein, ascorbic acid might be liberated as one of the by-products of the reaction. As yet, no evidence has been forthcoming to show that vitamin C exists in a combined form in the potato, but this, and other similar possibilities, must not be excluded.

It is of interest to note that Eidmann (1940) has patented a method whereby it is possible to segregate diseased from sound tuber seedlings. Slices of the tuber are immersed in an aqueous solution of a salt of selenious acid for 20-36 hours at 30° C. The different colourations produced, are noted. The diseased samples eventually turn a grey colour, while the healthy material stays at an intermediate pink stage.

The actual source of the ascorbic acid in the plant is rather obscure. It may be a direct product of photosynthesis, a by-product of photosynthesis or a degradation product of the plant's metabolism. Some evidence is available from the distribution and concentration of ascorbic acid through the plant's life-cycle.

It is certain that, when sprouting begins, ascorbic acid is travelling from the tuber to the sprout, but when the plant is actively growing in the field, the ascorbic acid content of the portions of the plant above ground is considerably in excess of that of the tubers, so that it would seem that in the leaves, or in other words at the seat of photosynthesis, we have the source of ascorbic acid. This is in agreement with the work of Giroud, Ratsimamanga and Leblond (1934 & 1935.2) who showed that vitamin C is present in much greater quantity in those parts of plants exposed to the light, than those parts kept in the dark, i.e. in the absence of chlorophyll. Similar results were obtained for comparable plants grown in the light and dark, and these workers suggest as is suggested here, that chlorophyll is necessary for the synthesis of vitamin C.

The concentration in the tuber is at its lowest level just when the initial sprouting stage is reached. During the growing season the concentration gradually increases in the newly formed tubers reaching a maximum just before maturity. This maximum is followed by a decrease throughout the dormant period until the following season when the whole process is repeated. Scheunert (1937) found that the concentration in the tuber was highest immediately after harvesting. This would seem to indicate that the ascorbic acid is only formed when the plant is actively growing.

Another point supporting this view is the variation of the ascorbic acid content of the leaves during the day. A maximum is reached in the early forenoon, and a minimum through the night which would seem to indicate that the synthesis of the vitamin is dependent on sunlight.

In the experiments with tubers sprouting in the light and in the dark, there was much more ascorbic acid present in the sprouts grown in the dark, though the actual concentration was less. The total amount of the vitamin present in the tuber and sprout after sprouting for three months was actually less than the amount present when the experiment was started. Growth was still taking place at the expense of the old tuber, the transition stage when the shoot begins to be able to synthesise the vitamin for itself not having been reached.

The probability is that the ascorbic acid is not produced directly by photosynthesis, but is derived from various sugars such as xylose, glucose or saccharose. According to Guha and Ghosh (1935), the addition of mannose to certain plant and animal tissues results in increased ascorbic acid content. This lends support to the theory that the vitamin is derived from sugars in the process of photosynthesis, and is not a direct product therefrom. The ascorbic acid in the leaves is probably a secondary or intermediate product from the synthesis of sugars, and in the tuber it is probably the result of

translocation.

An alternative point of view is that the vitamin may be a degradation product of the carbohydrate in the tuber, and passes to the leaves acting as a growth promoter to help in the process of photosynthesis. Ascorbic acid has been shown to occur in chloroplasts where the photosynthesis takes place, but this hypothesis is not very sound, since only the vitamin from the parent tuber would be available for the newly formed tubers, and experiment has shown that this is not so.

Summary.

The indophenol titration value has been shown to give a true representation of the ascorbic acid content of the potato tuber. Freezing results in a loss of ascorbic acid from the tuber. The vitamin C content of the growing plant varies considerably throughout the day and night, and also varies in the tuber with the time of storage. The amount of dehydroascorbic acid present remains fairly constant after one month's storage but increases, relative to reduced ascorbic acid, as the time of storage lengthens. When sprouting begins, there is a tendency for a local concentration of ascorbic acid to be set up at the base of the sprout. Manuring has very little effect, if any, on the vitamin content of the tubers. The incidence of virus disease produces, in many cases, an increase in the vitamin C content of the tubers, and suggestions are put

forward to account for this. The probable source of the ascorbic acid in the plant is also discussed.

Respiration of Potato Tubers.

Introduction.

The term respiration at first referred to the exchange of gases between the organism and its environment, but nowadays it is more usual to regard respiration as involving the whole of the katabolic process. Sachs, Pfeffer and Palladin who laid the foundation of all the work on plant respiration, all gave respiration this wider meaning. When respiration is so defined it becomes a much more fundamental property than the mere exchange of gases between organism and environment. It is a property of every living cell as well as of the whole organism. It must not be forgotten, however, that very few cases are known in which respiration does not involve an exchange of gases. Saussure (1797) made the first investigations of a quantitative character on the amounts of carbon dioxide evolved and oxygen taken up by various tissues. At this period there was a differentiation between diurnal and nocturnal respiration, and Liebig even denied that plants respired in a manner comparable to animals, but von Mohl (1851) made clear the difference between these two types of gaseous exchange, and from this time onwards, the term respiration ceased to be used in connection with the assimilatory process. Research dating from this period has been mainly concerned with the mechanism of the process, involving work on the

nature of the substrates, the various stages in the respiration process and its relation to the enzymes present in the cell.

Respiration in its commonest form implies the oxidation of organic material, such as carbohydrates and proteins, for which a supply of oxygen is necessary. This is the aerobic or normal mode of respiration of plants, and is the only one which need concern us here, but an essential property of all respiration is the release of energy. Most of this energy is dissipated in the form of heat, and only a small proportion is transformed into mechanical or chemical energy. The respiratory process is not constant throughout the plant kingdom, and its essential characteristic release of energy may be effected in various ways. The outward sign of the process is the consumption of oxygen and the elimination of carbon dioxide. Theoretically, respiration could be studied quantitatively by determining either the oxygen consumption or the carbon dioxide evolution of the respiring tissue. In practice, the determination of one or other of these quantities usually forms the basis of respiration measurement.

In the present state of biochemical knowledge, little is accurately known of the differences in respiration between various types of cells, and much less of the respiration of one part as contrasted with another part of the same cell.

Thus, any discussion on respiration must concern itself more with the organisms as chemical reagents or material, at least, containing such reagents. This state of affairs is rather unfortunate, but biochemistry has not yet advanced far enough to enable one to describe the cell as a whole.

Mechanism of Respiration.

Respiration takes place over the whole plant and is independent of light and chlorophyll. For the simplest state of affairs we may assume the substrate to be a carbohydrate which, on being oxidised to carbon dioxide and water, involves the consumption of a volume of oxygen equal to that of carbon dioxide evolved according to the general equation -

$$C_xH_{2y}O_y + xO_2 = xCO_2 + yH_2O$$

In many cases, however, the volume of oxygen absorbed and the volume of carbon dioxide evolved are not the same. In the example given above, the complex molecule is assumed to break down in one stage, but this is extremely improbable and the nature of the various stages of the break-down of the carbohydrate is of primary importance. Further, under the temperature conditions prevailing in the growing plant, no break-down of carbohydrate into carbon dioxide and water takes place if we simply supply carbohydrate with oxygen. Some systems, enzymatic or otherwise, inside the cell, must have a connection with the respiratory process. It is indeed a

possibility that every stage in the process is catalysed by an enzyme. Little direct evidence is available on the mechanism of the respiration process, but a considerable amount of indirect evidence is available from fairly recent investigations on yeast fermentation. It does not follow that, because the end products and substrates of two or more processes are the same, the mechanism of the reactions is the same, but there is, however, a certain amount of evidence which suggests that this is indeed so. (Stoklasa and Czerny (1903)). Pfeffer (1878) suggested that ordinary aerobic respiration takes place in two stages. (1). The splitting of sugar into alcohol and carbon dioxide and (2) the oxidation by atmospheric oxygen of the alcohol into carbon dioxide and water. This theory found little support and indeed Pfeffer himself ultimately gave it up. Recently, it has found favour again, but not in the simple form in which Pfeffer conceived it. The newer idea is that, the alcohol fermentation takes place in several stages with the formation of labile intermediate substances produced by enzyme action preceding the production of alcohol. Acetaldehyde might be such an intermediary. Jensen (1923) criticised this hypothesis on the grounds that, in certain organisms, the ratio of the rate of anaerobic to the rate of aerobic respiration sinks below 1/3 without the material suffering damage. In such cases anaerobic processes do not split up enough

sugar to account for the whole of the aerobic respiration.

It is now assumed that Y-fructose usually forms the substrate for respiration, though it is not necessary for it to be present as such in the plant in the first instance. The chief enzymes associated with the end products of respiration are oxidase, peroxidase, catalase, zymase and carboxylase.

The course of aerobic respiration may be conveniently divided into three main parts, though each part may contain several stages.

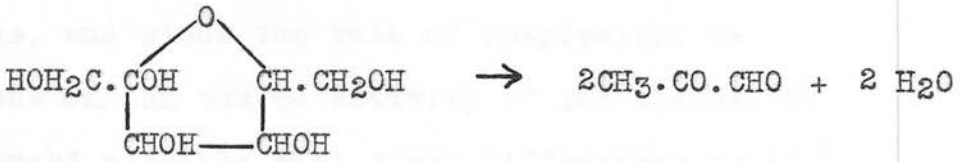
(1) The production of active hexose (Y-fructose) from the normal sugar or other material used in respiration.

(2) The breaking down of the active hexose by means of enzymes of the zymase complex.

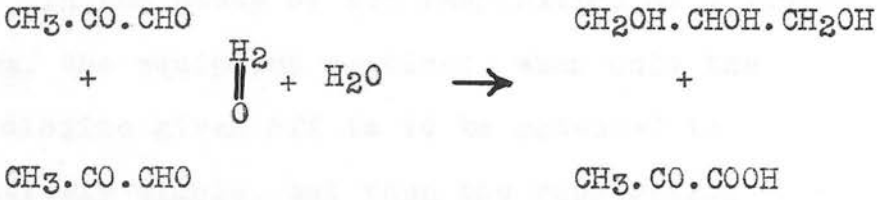
(3) The oxidation of the intermediate products formed in (2).

The work of Neuberg and co-workers (1918, 1921, 1924, 1925 and 1926) has rendered it almost certain that acetaldehyde is an intermediate product of respiration. Neuberg has suggested a series of reactions by which the sugar may be broken down to acetaldehyde and which has a working hypothesis. These stages are as follows :-

(1) The splitting of the hexose molecule into two molecules of methyl glyoxal with the removal of water.



(2) A Cannizzaro reaction now follows in which two molecules of methyl glyoxal are concerned, one being reduced to glycerol, the other oxidised to pyruvic acid.



(3) The pyruvic acid is now split into acetaldehyde and carbon dioxide.

The last stage in aerobic respiration is the oxidation of acetaldehyde or some other intermediate product by means of one of the oxidation mechanisms present in the plant. The actual course of this stage is still very obscure. Neuberg's scheme agrees very closely with that put forward by Blackman (1928) who worked on the respiration of apples. From all this it is very obvious that enzymes hold a place of paramount importance in respiration measurements, and it was because of this that respiration measurements were undertaken in the present investigation.

Previous work by Joszt and Starezewski (1922) and Lauder and Robertson (1931) had suggested that there were varietal differences in the enzyme content of potatoes, and since the rate of respiration is dependent on the enzyme activity of the tissue, it was thought possible that these differences would be evident if the respiration rates of several varieties could be measured.

Practical Considerations.

In the study of the respiration of a living organism, the equipment required, when only the carbon dioxide given off is to be measured is comparatively simple, but when the respiration quotient (CO_2/O_2) is to be measured, some means of determining the uptake of oxygen, as well as the carbon dioxide produced, must be provided. The apparatus described here is based on a well-known basic principle. Several modifications have been introduced, however, to simplify the working of the system. Temperature plays an important part in the respiration intensity, an increase in temperature usually giving an increase in the rate of respiration. Light in itself has no effect on the respiration, but it may cause organic decomposition giving rise to the liberation of carbon dioxide. According to Blackman (1928) the carbon dioxide production varies with every concentration of the oxygen environment. Increased concentrations of carbon dioxide also bring about a very marked depression in the rate

of respiration. Böhm (1887) and Stich (1891) showed that, when potatoes were cut, the respiration was increased. Later, Richards (1896) showed that the increase was dependent on the extent of the wounding. When a potato was cut, there was a large increase in the rate of respiration in the first two or three hours followed by a rapid decrease, due to the escape of gases previously enclosed within the tissue.

From the above considerations, it is obvious that when the respiration rate is being measured, the following factors must be taken into account.

(1) The temperature must be kept constant.

(2) Light should be excluded.

(3) The concentration of oxygen should be kept as constant as possible.

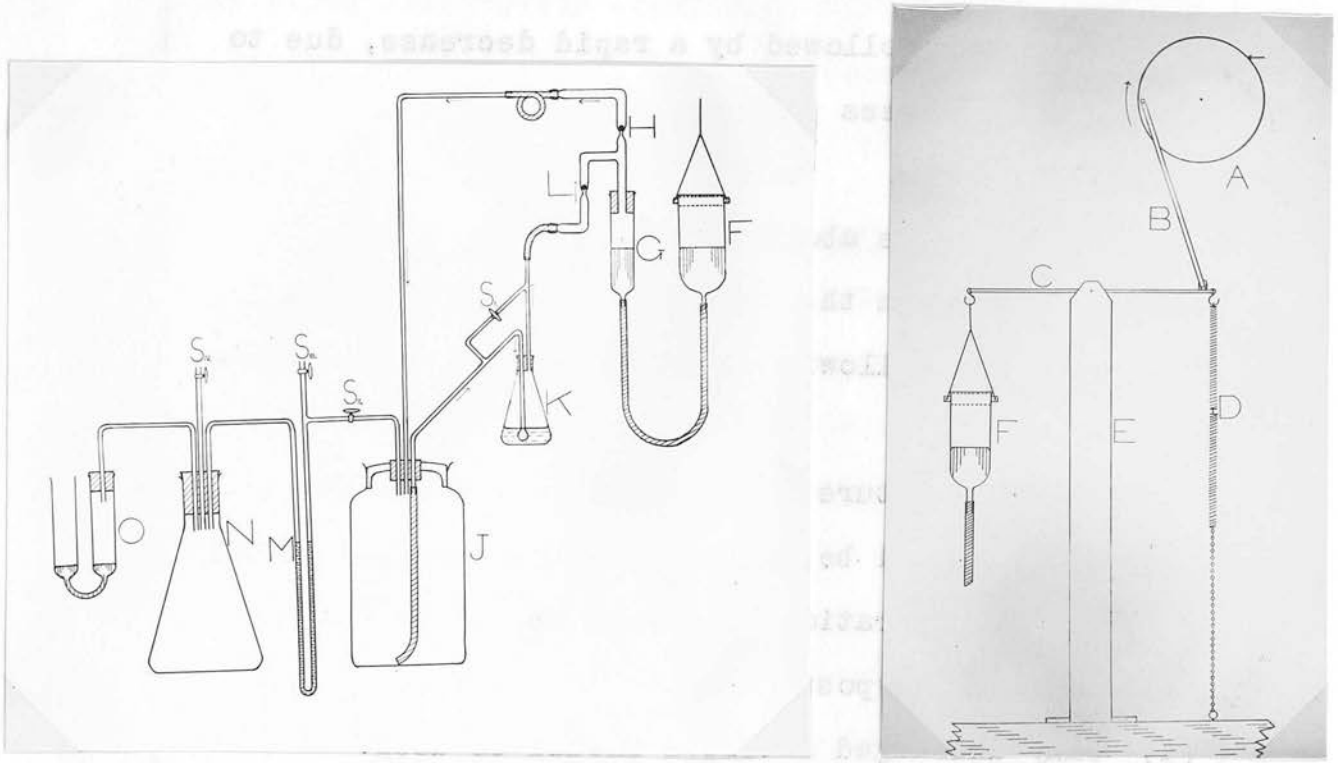
(4) Only undamaged material should be used.

The apparatus to be described here was developed from the gas circulating pump of Leach (1930) and from the Pettenkoffer method of Hanes and Barker (1931).

Description of the Apparatus.

The attached diagrams and photographs will illustrate the mode of assembly and working details of the apparatus. In the diagrams all but the connecting tubes is drawn to scale.

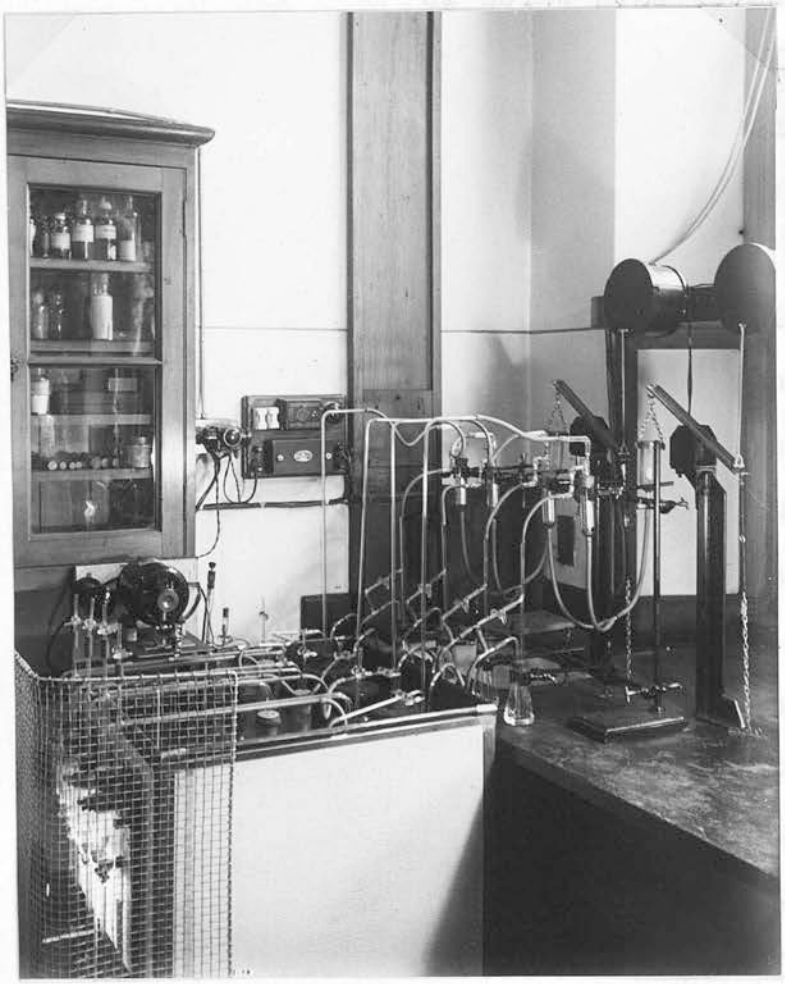
About 1 kg. of potato tubers is placed in the container J, capacity $2\frac{1}{2}$ litres, which is a



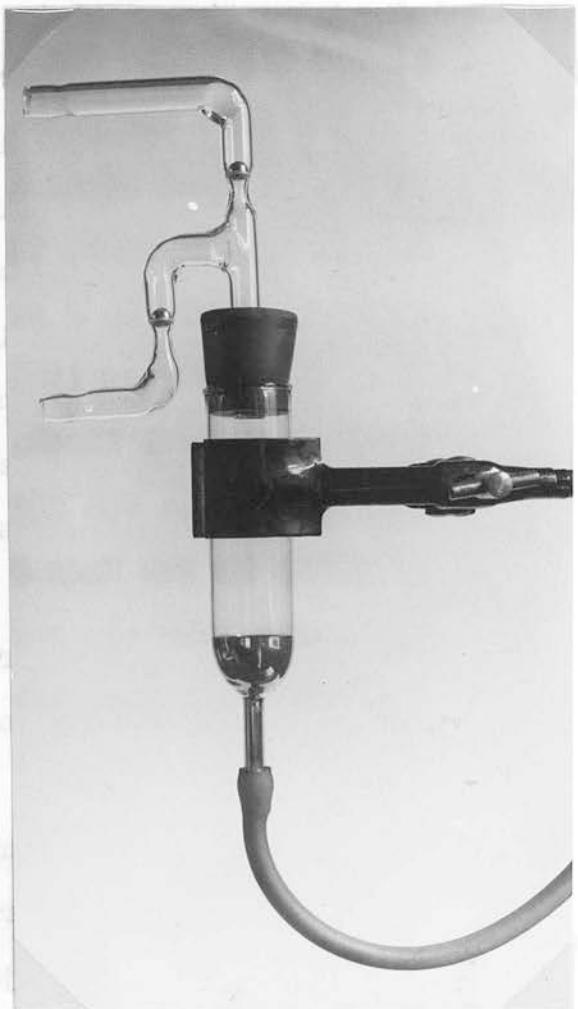
Schematic Diagram of Respiration Apparatus.

large bottle with a hole to take a 6A rubber stopper cut in the ground-in glass lid. This container is connected in series with a water manometer M, compensating flask N and mercury manometer O, of the same diameter as G. The container and compensating flask are immersed in a water bath which is thermostatically controlled at 20° C. ± 0.2° C. by means of a mercury-toleune regulator working a hot-wire relay switch for an electric immersion heater.

To keep the air circulating in J the pump G is worked from a small electric turntable A, with an induction motor, moving at 2 r.p.m. A crank B makes connection between the periphery of A and one end of the fulcrum C which is supported on a stand of angle iron E. The mercury reservoir F is attached to the other end of the fulcrum. When the system is in motion, there is a continuous change in the weight of the reservoir and the difference at opposite ends of the stroke is too much for the motor to overcome. The reservoir is, therefore, balanced by the springs D whose tension is adjusted by varying the number of links of chain anchored to the bench. The reservoir works a pair of pumps like that shown in G. By means of the springs and by having the rubber connections as short as possible, the up and down movement of the reservoir is perfectly regular and puts little strain on the motor. The pump G is made from Pyrex glass, 12 mm. internal diameter, with 1/4" stainless steel balls lightly ground in at the



General View showing arrangement of Motors and Pumps.



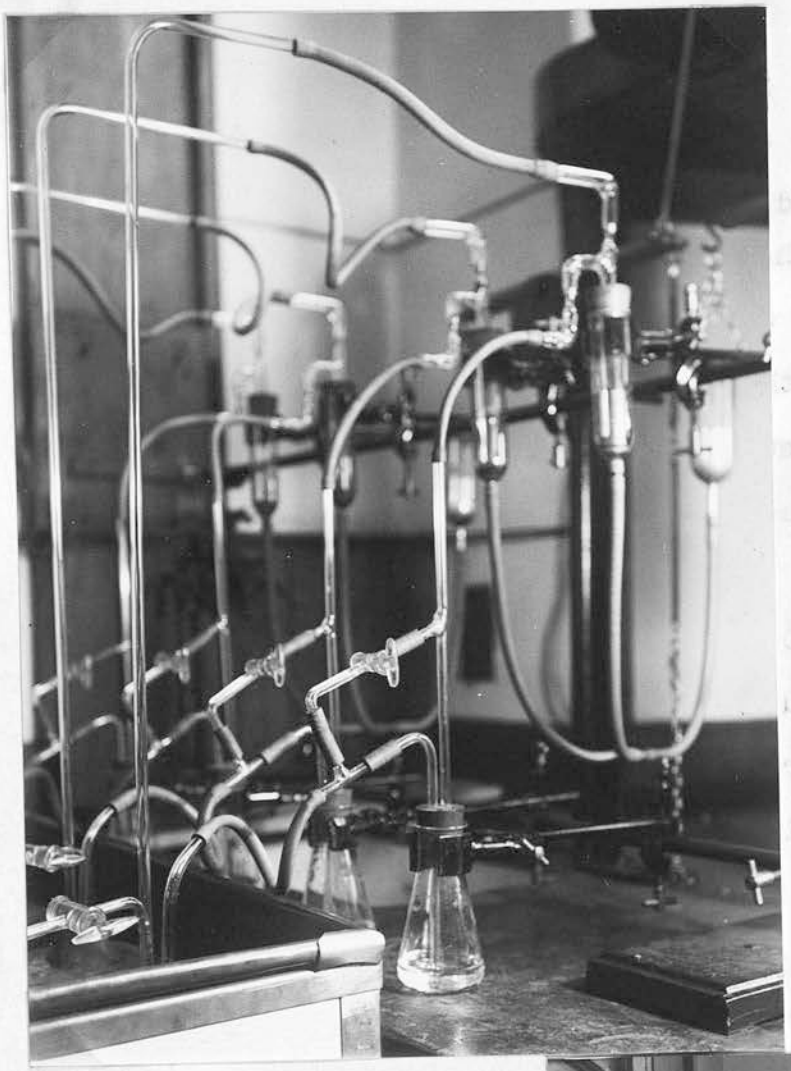
Close-up of Pump.

constrictions H and L, and the whole is so arranged that it can be easily dismantled and the balls removed for cleaning when necessary. As the mercury rises in G the air is forced past the valve H and into the top of the container; as the mercury falls in G the air is sucked through L from the bottom of the container via the absorbing solution in the flask K into the pump. The capacity of G is about 40 ml. so that about 80 ml. passes through K per minute and there is thus approximately three complete changes of air in J per hour.

The tube leading into K, a 100 ml. wide-mouthed conical flask, has a bulb pierced with small holes, so that the air passes into the absorbing solution in the form of very small bubbles. The absorbing solution consists of a definite volume of N/10 NaOH and the carbon dioxide is determined by adding excess barium chloride and titrating the excess hydroxide as in Winkler's method.

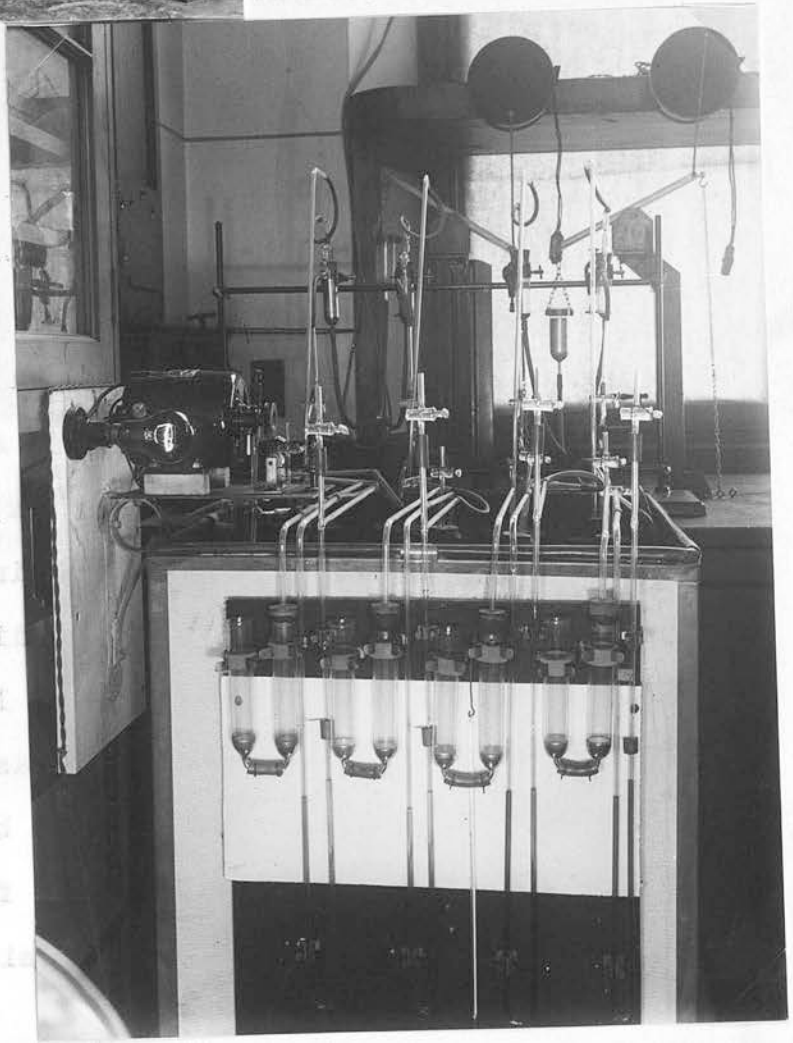
During the experiment, the apparatus is kept air-tight by means of a liberal application of a proprietary sealing compound called Apiezon "Q".

In the actual investigation, four sets of apparatus were used, the four containers and their respective compensating flasks standing on a wire tray in a copper tank about 2 ft. cubed, lagged on the outside with asbestos. The tank was also fitted with a stirrer, the speed of which could be varied at will. The manometers M which were about 2 ft. long were all attached to a board mounted on the side of the tank



General View showing Pumps and Absorption Flasks.

General View showing Manometers.



as will be seen in the photographs. The compensating flask should have a volume equal to the volume of air left in the containers after the potatoes are put in. Various liquids such as coloured alcoholic and glycerine solutions were tried in the manometers, but the one finally decided upon was a dilute solution of methylene blue in water. On one limb of the manometer, a pointer was attached so that the level of the liquid could be noted. The absorption flask K was by-passed by a capillary tube, so that pressures could be equalised when readings were being taken.

Use of the Apparatus.

The tubers to be used for the experiments were kept at a low temperature of about 5° C. till required for use. They were then taken into the laboratory and carefully washed and dried and stored for 24 hours at 20° C. About eight or nine undamaged tubers weighing 800-1000 g. were taken for each determination and placed in the containers with the exit tube carried right to the bottom, and the lids sealed in with Apiezon "Q". The absorption flasks K were next put in position. For the first hour the flasks contained 25 ml. 10% NaOH to absorb the initial CO_2 present. After this time the flasks were replaced by others containing 25 ml. N/10 NaOH. Great care is necessary to see that all the apparatus is air-tight.

A determination is commenced at atmospheric pressure, all four stopcocks S being open and the mercury level in G fixed at a particular point. (A needle in the stopper of G and a mark on the turntable A serve as checks on this level). Stopcocks S_1, S_{111}, and S_{1v}, are then closed. When it is desired to measure the amount of oxygen absorbed, S_1 is opened to equalise pressures in the system, the mercury in G is brought back to the same point as at the commencement, and the liquid in the two limbs of the manometer M adjusted to their original levels by admitting oxygen through the stopcock S_{111} from a gas burette.

The volume of carbon dioxide given off over a given time can be calculated and the corresponding volume of oxygen used being known, the respiration quotient can be calculated.

In the present investigation, both healthy and diseased material was used as well as material from different sources. Each determination was normally allowed to run for four hours. Since respiration is connected with enzyme activity, it was thought probable that a connection could be shown between the rate of respiration or the carbon dioxide output and the enzyme activity. Lauder and Robertson (1931) have given figures for the tyrosinase activity in potatoes, and these figures were used in the results to follow. Most of the respiration experiments were carried out after four months' storage, and the figures for the ascorbic acid

content after four months' storage were also used for comparison. The results are given in Tables 21 - 24 and Graph 4.

GRAPH 4.

RELATION BETWEEN ASCORBIC ACID CONTENT AND TYROSINASE VALUE AND WT. OF CO_2 EVOLVED.

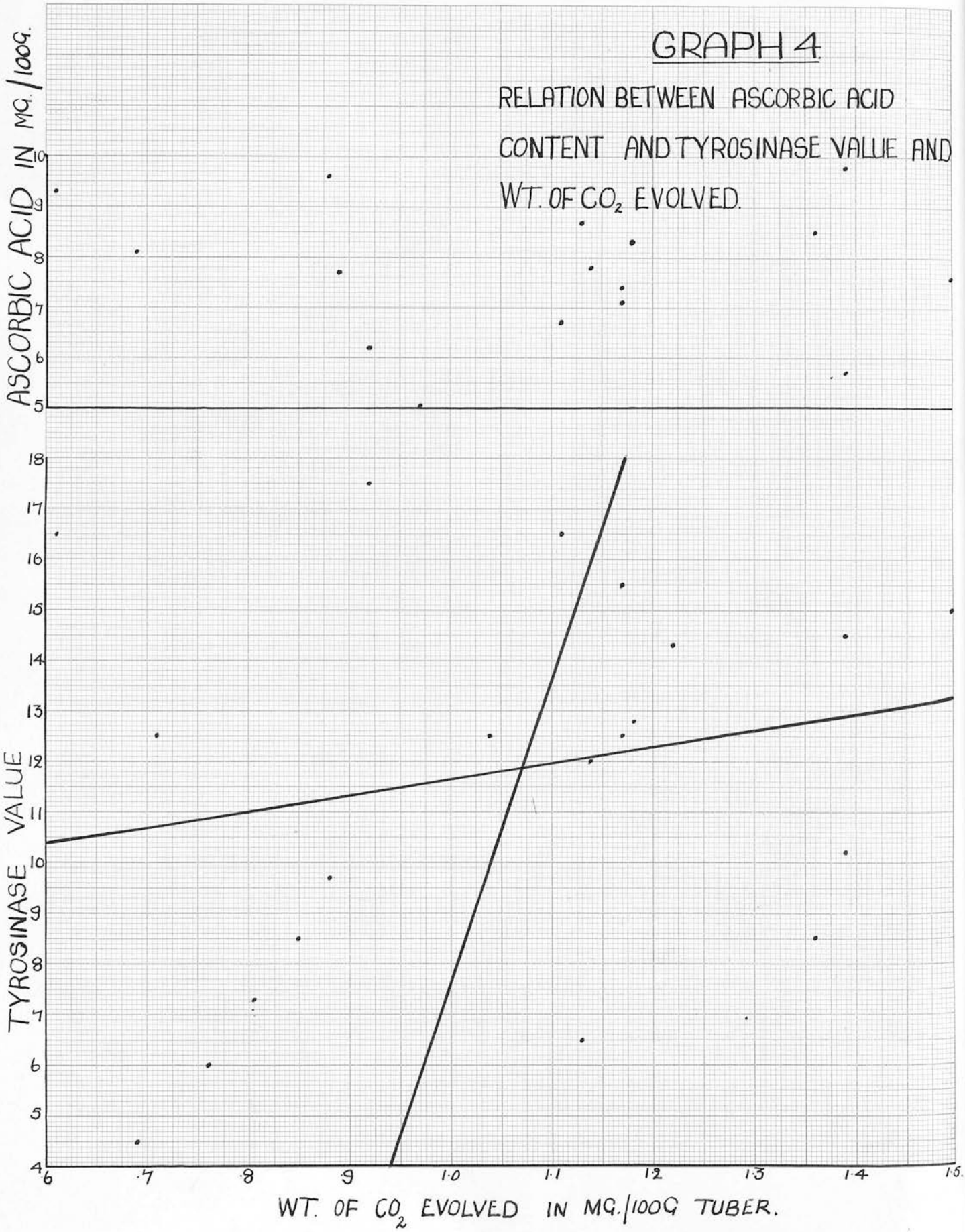


Table 21.

Wt. of CO_2 evolved per hr. in mg. per 100 g. tissue from tubers from different sources compared with the Tyrosinase Activity and the Ascorbic Acid Content.

Variety	Tyr.	Vit.C	E.H.	M.	A.	D.	T.
Di-Vernon	17.5	6.2	0.92	-	-	-	-
President	16.5	6.7	1.11	-	-	-	-
Gladstone	16.5	9.3	0.61	0.85	0.70	1.15	-
Dunbar Cavalier	15.5	7.1	1.17	0.79	0.80	-	0.80
Doon Star	15.0	7.6	1.53	1.01	1.01	1.01	-
Golden Wonder	14.5	9.8	1.39	0.89	1.05	1.54	1.08
Immune Ashleaf	14.3	-	1.22	-	1.12	1.01	-
Duke of York	12.8	8.3	1.18	0.93	0.86	0.89	-
Majestic	12.5	7.4	1.17	0.87	0.75	0.84	-
Catriona	12.5	-	1.04	-	-	-	-
Arran Pilot	12.5	-	0.71	0.88	0.89	1.10	1.06
Epicure	12.0	7.7	1.14	0.93	0.95	1.15	0.98
Arran Chief	10.2	5.7	1.39	-	-	-	-
Redskin	9.7	9.7	0.88	0.91	0.92	1.12	-
Great Scot	8.5	-	0.85	0.99	1.07	1.18	1.18
Up-to-Date	8.5	8.5	1.36	-	-	-	-
Arran Banner	6.5	8.7	1.13	1.02	0.94	0.95	-
Kerr's Pink	6.0	-	0.76	0.88	0.80	1.03	-
Eclipse	5.5	-	-	0.91	1.03	1.25	-
King Edward	4.5	8.1	0.69	0.95	0.87	0.96	0.96
Dunbar Standard	-	7.7	0.89	-	0.89	0.95	0.90
Arran Consul	-	4.9	0.97	-	-	-	-
Sharpe's Express	-	-	1.00	-	-	-	-
Ninetyfold	-	-	-	1.10	0.91	1.05	1.22
Average			1.05	0.99	0.91	1.06	1.02

Tyr. = Tyrosinase Activity, See Text.

Vit. C = Ascorbic Acid Content in mg. per 100 g. tissue.

E.H. = East Craigs.

A. = Findlay, Aberdeen.

M. = McAlister, Perth.

D. = Dobbie, Edinburgh.

T. = Thyne, Dundee.

Table 22.

Wt. of CO_2 evolved per hr. in mg. per 100 g. tissue from healthy and diseased tubers.

Variety	E.H.	E.S.M.	E.L.R.
Gladstone	0.61	-	0.94
Dunbar Cavalier	1.17	1.06	1.41
Doon Star	1.53	1.32	1.29
Golden Wonder	1.39	1.38	1.28
Immune Ashleaf	1.22	1.26	1.32
Duke of York	1.18	1.20	-
Majestic	1.17	0.78	1.34
Arran Pilot	0.71	1.15	0.88
Epicure	1.14	-	1.20
Arran Chief	1.39	1.02	1.74
Redskin	0.88	1.16	-
Great Scot	0.85	0.94	1.80
Up-to-Date	1.36	0.82	1.09
Arran Banner	1.13	0.86	0.89
Kerr's Pink	0.76	0.75	1.26
King Edward	0.69	1.10	1.04
Arran Consul	1.00	1.24	1.22
Average	1.07	1.07	1.24

Table 23.

Respiration Quotient of Healthy and Diseased Tubers.

Variety	E.H.	E.S.M.	E.L.R.
Gladstone	1.34	-	0.74
Dunbar Cavalier	0.82	1.43	0.89
Doon Star	1.01	0.72	0.78
Golden Wonder	-	0.83	0.96
Immune Ashleaf	-	0.90	0.89
Duke of York	1.47	1.25	-
Majestic	0.55	1.17	1.77
Arran Pilot	-	0.75	0.87
Epicure	1.07	0.97	-
Arran Chief	-	0.91	-
Redskin	-	0.85	-
Great Scot	-	1.10	0.84
Up-to-Date	1.01	0.99	0.60
Arran Banner	2.08	1.43	1.06
Kerr's Pink	-	0.67	2.05
King Edward	1.25	1.01	-
Arran Consul	1.53	0.82	1.77
Average	1.22	0.99	1.16

Table 24.

Respiration Quotient of Tubers from Different Sources.

Variety	Tyr.	Vit.C	E.H.	M.	A.	D.	T.
Di-Vernon	17.5	6.2	1.01	-	-	-	-
President	16.5	6.7	1.90	-	-	-	-
Gladstone	16.5	9.3	1.34	0.68	0.79	0.62	-
Dunbar Cavalier	15.5	7.1	0.82	1.22	-	1.13	1.29
Doon Star	15.0	7.6	1.01	0.98	0.92	0.79	-
Golden Wonder	14.5	9.8	-	0.89	1.05	1.54	1.08
Immune Ashleaf	14.3	-	-	-	1.41	1.01	-
Duke of York	12.8	8.3	1.47	0.72	0.86	0.82	-
Majestic	12.5	7.4	0.55	1.38	2.47	0.74	-
Catriona	12.5	-	0.98	-	-	-	-
Arran Pilot	12.5	-	-	1.42	0.86	0.79	0.75
Epicure	12.0	7.7	1.07	0.57	0.91	0.78	0.85
Arran Chief	10.2	5.7	-	-	-	-	-
Redskin	9.7	9.7	-	1.14	0.65	0.99	-
Great Scot	8.5	-	-	0.52	1.06	0.90	0.68
Up-to-Date	8.5	8.5	1.01	-	-	-	-
Arran Banner	6.5	8.7	2.08	1.07	1.21	1.00	-
Kerr's Pink	6.0	-	-	0.54	0.42	1.10	-
Eclipse	5.0	-	-	0.92	0.83	1.16	-
King Edward	4.5	8.1	1.25	0.84	0.87	1.22	0.92
Dunbar Standard	-	7.7	-	-	0.69	0.73	0.89
Arran Consul	-	4.9	1.53	-	-	-	-
Sharpe's Express	-	7.9	1.23	-	-	-	-
Ninetyfold	-	-	-	0.74	0.75	1.04	0.85
Average			1.23	0.91	0.92	0.96	0.94

Discussion of Results.

There is very little obvious relation between any of the results for any particular variety when taken individually. When the carbon dioxide evolution of the tubers from the different sources (Table 21) is averaged, the agreement is not very good, but when the average respiration quotient for the same sources (Table 24) is considered, the East Craigs' tubers give a much higher value than the other four sources which show very good agreement among themselves.

The effect of disease on the carbon dioxide output (Table 22) is quite marked. Tubers infected with mosaic gave an identical value with the healthy material, but the average value for tubers infected with leaf-roll is markedly increased. This, however, is not shown when the respiration quotients are averaged (Table 23). Whitehead (1934), who measured the carbon dioxide evolution of healthy and leaf-roll infected plants found that, throughout the greater part of the life-cycle, the weight of carbon dioxide evolved from the diseased plants was greater than that from the healthy plants.

The weight of carbon dioxide evolved seems to give more consistent results than the respiration quotient, so it was decided to plot the carbon dioxide evolved against the tyrosinase value and the ascorbic acid content (Graph 4). The points are very scattered, and there is no clear-cut correlation.

In the case of ascorbic acid, the scattered diagram (Graph 4) shows that there is no correlation between the ascorbic acid and carbon dioxide evolved. The scattered diagram for tyrosinase and carbon dioxide suggests a general tendency for a positive correlation, but the correlation co-efficient calculated for the two sets of values is only plus 0.23 which, for 19 observations, does not reach the level of significance. The regression lines have been inserted in the diagram.

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